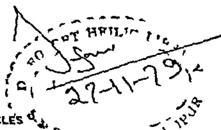
VOL 68 / JAN 1979 / NO 1

ACTA PÆDIATRICA SCANDINAVICA

ISSN 0001-656X



CONTENTS

ORIGINAL ARTICLES

Continued on cover page 4

DISTRIBUTED BY THE ALMQVIST & WIKSELL PERIODICAL COMPANY STOCKHOLM SWEDEN

ACTA PÆDIATRICA SCANDINAVICA

Subscription and Distribution

The Almqvist & Wiksell Periodical Company
26 Gamla Brogatan
S 101 20 Stockholm 1 Sweden

Acta Pædiatrica Scandinavica publishes papers in lish on scientific investigations in the field of paedi Each issue consists of about 100 pages six nu forming one volume The numbers will be issued second month. *Supplements* will be delivered to scribers free of charge

Acta Pædiatrica Scandinavica will consider p from authors in all countries Articles are acc for publication on condition that they are contri solely to this journal and have not been publ elsewhere previously Original articles should r scientific findings within the paediatric field of int Review articles should contain an objective ac of different points of view and an internatio representative bibliography Case reports should tain accounts of rare syndromes new diagnostic and methods, new kinds of treatment and labor research with foreseeable practical application L to the Editor is intended to be a forum for comr on current literature short discussions and preliminary communications which are thought t important and urgent enough to warrant rapid lication

All papers are subject to editorial revision to i them conform to the style adopted by Acta Pædi Scandinavica A paper should not exceed 6 pr pages including space for figures and tables E tions are occasionally allowed but the author wi charged for the exceeding pages Tables and illu tions covering more than three pages in original an and two pages in Case reports are also printed a author's expense Monographs and more exte articles may be published separately as Supple at the author s own expense

ACTA PÆDIATRICA SCANDINAVICA

Vol 68 1979

Contents

Original Articles

Review Article

Case Reports

Short Communications

Letters to the Editor

Supplements

Review Article

Case Reports

Short Communications

Subject Index

General Index

Authors Index

Subject Index

Acta Pædiatr Scand 68 1–9 1979

SUDDEN INFANT DEATH IN COPENHAGEN 1956–1971

II *Social Factors and Morbidity*

FIN BIERING SØRENSEN TORBEN JØRGENSEN and JØRGEN HILDEN

From the Municipal Agency of Infant Health Visitors Copenhagen and the Institute of Human Genetics University of Copenhagen Copenhagen Denmark

ABSTRACT Biering Sørensen F Jørgensen T and Hilden J (Municipal Agency of Infant Health Visitors Copenhagen and Institute of Human Genetics University of Copenhagen Copenhagen Denmark) Sudden infant death in Copenhagen 1956–71 II Social factors and morbidity Acta Paediatr Scand 68 1 1979.—131 cases of the Sudden Infant Death Syndrome (SIDS) among infants born in the Municipality of Copenhagen during 1956–71 were analysed on the basis of data collected prospectively by the infant health visitors and abstracted from police reports Compared with controls a significantly larger number of SIDS infants had been born out of wedlock were living only with their mother had parents in a less secure occupation lived in more crowded poor-quality dwellings and districts The home standard was lower among the SIDS families including a lower standard of infant care a higher percentage of not keeping the appointments with the infant health visitors and a lower mental capacity in the mothers there was no difference with respect to the mother s physical capacity More congenital malformations and more cases of asphyxia were found among the SIDS infants and a significantly larger number of the SIDS infants had been admitted to hospital with a tendency to being kept there longer It is concluded that a relationship exists between poor social conditions increased morbidity and SIDS At the same time it is pointed out that during recent years the differences between cases and controls as regards certain of the social parameters studied have decreased

KEY WORDS Sudden infant death socioeconomic factors infant care morbidity

The Sudden Infant Death Syndrome (SIDS) and its relationship to certain social parameters have been known for many years (1 8 17) The present paper is intended to shed more light on these aspects as well as the morbidity of the SIDS victims using prospectively collected data It forms part of a larger epidemiological study of SIDS in the Municipality of Copenhagen (3 10 11)

MATERIAL AND METHODS

SIDS is defined here as The sudden death at home of infants between one week and one year which is unexpected by history and in which a thorough post mortem examination fails to demonstrate an adequate cause for dea h (3)

The material comprises all 139 SIDS victims born in the Municipality of Copenhagen during the period 1956–71 (0 9 SIDS cases per 1000 live births) Age at death ranged from 10 to 359 days (median 1?0 days mean 13 days) (3) *Police reports* were available for a l these cases

In 131 of the cases (44 females and 87 males) prospectively collected data were obtained from the case records filed by the *infant health visitors* in the Municipality of Copenhagen (3) Each of these 131 SIDS cases was matched by the aid of the infant health visitors files with four controls living at home and with a few exceptions of the same sex and birth date (3) Among the SIDS cases (hereinafter called cases) there were 26 (19 8%) prematures (birth weight ≤2 500 g) and among the 5?4 controls 16 (3 1%)

Data concerning symptoms or illness during the last 4 weeks before death are derived from the police reports All other results i e information concerning social factors and morbidity are from data collected by the health visitors

During the study period the population of Copenhagen Municipality fell from 746 034 in 1956 to 625 678 in 1971 The number of live born infants was 9 817 in 1956 and remained at this level until 1963 when it rose to 10 568 where it remained until 1966 It then dropped to 9 690 in 1967 and fell steadily thereafter to 7 751 in 1971

In order to detect temporal trends the time under investigation was divided into four year periods as follows unless otherwise stated 1956–59 1960–63 1964–67 and 1968–71 It should be noted that the third four year period is characterized by a high birth rate reflecting the fact

Table 1 *Percentage (and number) of cases and controls born in and out of wedlock (left) and living with their parents or only with their mother (right)*

	Born		Living with	
	In wedlock	*Out of wedlock*	*Both parents*	*Only the mother*
Cases	76 3 (100)	23 7 (31)	82 9 (107)	17 1 (22)
Controls	93 1 (488)	6 9 (36)	95 4 (500)	4 6 (24)
χ^2 (d f)	30 39 (1)		22 73 (1)	
p value	<0 0001		<0 0001	

that the approx 20-year-olds born during the high birth rate years around 1945 were then having their firstborns This factor was taken into consideration in evaluating temporal trends

The chi square and Mann Whitney rank sum tests were used in the statistical analysis with 5% as significance level

RESULTS

Civil status

A significantly larger number of cases than of controls were born out of *wedlock* and a larger number of cases were *living with* their mother alone (Table 1) The number of cases as well as controls living with both parents exceeded that of those born in wedlock due to the considerable number of unmarried couples living together The values in the table have been *sub divided* by sex but there was no difference Division into prematures and matures also showed no difference in the trend Subdivision of the material into the named four year periods revealed nothing apart from the general tendency to fewer infants born in wedlock

Parents occupation

The parents occupation is recorded by the infant health visitors at the first visit the mother s as that up to the possible childbirth leave

Table 2 shows no significant difference in *mother s occupation* between cases and controls When regarding only mothers who go out to work there are significantly more salaried employees (including civil servants) among the control mothers, whereas the case mothers were more often wage earners (χ= 3 88 d f =1 p=0 049) Subdivision into the four-year periods showed no definite differences in the pattern except for the increased tendency to go out to work

As to mothers living alone with their infants (22 and 24 in the last column of Table 1) there is no difference with respect to occupation In both groups only 20% did not go out to work

Table 3 sets out the *father s occupation* when the baby was living with both parents There is a significantly larger number of salaried employees in the control group and more wage earners in the SIDS group From 1956–59 to 1968–71 there was a tendency for this difference between the case and control group to diminish the percentage of salaried em

Table 2 *Percentage (and number) of cases and controls whose mothers did not go out to work were salaried employees or were wage earners*

Mother s occupation	Cases	Controls
Did not go out to work	69 4 (84)	66 7 (326)
Salaried employees incl civil servants	19 0 (23)	26 4 (129)
Wage earners	11 6 (14)	7 0 (34)
χ^2 (d f)	4 85 (2)	
p value	0 088	
Other occupations	(10)	(30)
Unknown	(0)	(5)
Did not go out to work 1956–59	87 5 (28)	88 3 (113)
Did not go out to work 1968–71	37 9 (11)	35 3 (41)

Table 3 *Percentage (and number) of cases and controls whose fathers were salaried employees or wage earners provided the infant was living with both parents*

Father s occupation	Cases	Controls
Salaried employees incl civil servants	20 4 (19)	38 1 (158)
Wage earners	79 6 (74)	61 9 (257)
χ^2 (d f)	9 65 (1)	
p value	0 0019	
Other occupations	(8)	(71)
Unknown	(6)	(14)

ployees in the control group remaining practically unchanged (about 38%) while in the SIDS group it rose from 17% to 28%

Neither the data underlying Table 2 nor those of Table 3 showed any sex difference

In Tables 2 and 3 the term other occupations indicates self employed and liberal They were excluded from the present calculations partly because these occupations are very inhomogeneous and partly because the groups are relatively small

Salaried employees in general may be said to have a more secure occupational status than wage earners This is primarily because the former have one to three months notice whereas the wage earners often can be sacked at day s notice Besides the members of the salaried employees group generally have better education and possibilities of earning more When considering the *occupation of cohabiting parents jointly* it is possible therefore to set up more or less socially secure occupational combinations It was found that 22 6% of the cases and 41 4% of the controls had parents in a socially more secure occupational combination characterized by the father being a salaried employee and the mother also a salaried employee or not going out to work Correspondingly 77 4% of the cases and 58 6% of the controls had parents in a socially less secure occupational combination the father being a wage earner and the mother a wage earner or not going out to work This aspect differed significantly for the cases and controls (χ^2=9 41 d f =1 p=0 0021)

Housing

In Table 4 the *districts of the infant health visitors* in the Municipality of Copenhagen are divided into above average average and below average This classification was made by the head of the Municipal Agency of Infant Health Visitors by informally estimating the housing and the social composition and standard of the districts but not knowing the figures of the present study It should be mentioned here that the residential quarters of the well to-do are almost entirely outside the Municipality of Copenhagen It is apparent from the table that a significantly larger number of cases than of controls lived in the below average districts There was no sex difference and there was no definite trend to a change in the difference through the years

Table 5 shows that the *person/room ratio* was significantly higher in the case group than in the control group There was no sex difference Apart from a general reduction of this ratio from 1956–59 to 1968–71 there was no definite change in the pattern evident from Table 5

The quality of the dwelling (Table 6) was as

Table 4 *Percentage (and number) of cases and controls who lived in different districts in the Municipality of Copenhagen classified as above average average and below average*

Above average Østerbro Brønshøj Vanløse and Valby average City Amager North West Sydhavnen and the neighbouring part of Vesterbro *below average* Islands Brygge Christianshavn Nørrebro and the remaining part of Vesterbro

Districts	Cases	Controls
Above average	28 1 (36)	38 8 (202)
Average	37 8 (47)	36 9 (192)
Below average	39 1 (50)	24 4 (127)
χ (d f)	11 75 (2)	
p value	0 0027	
Unknown	(3)	(3)

Table 5 *Percentage (and number) of cases and controls by number of persons per room*

Persons per room (P/R)	Cases	Controls
(P/R)≤1	5 7 (7)	15 6 (78)
1<(P/R)≤2	74 6 (91)	71 3 (356)
2<(P/R)	19 7 (24)	13 0 (65)
χ^2 (d f)	10 16 (2)	
p value	0 0062	
Unknown	(9)	(27)

Table 6 *Percentage (and number) of cases and controls by quality of dwelling as classified by the infant health visitor*

Quality of dwelling	Cases	Controls
Above average	17 7 (22)	31 4 (161)
Average	56 5 (70)	60 7 (311)
Below average	25 8 (32)	7 8 (40)
χ^2 (d f)	35 37 (2)	
p value	<0 0001	
Unknown	(7)	(12)

sessed by the infant health visitors The classification into above average average and below average was based on whether the dwelling was dark, humid without a separate kitchen bathroom or toilet plus its lay out and maintenance on the whole On the other hand this evaluation paid no regard to the number of rooms and persons It applies to the data in Tables 6–9 that all are based upon a personal estimate and that the manner of estimating has changed somewhat in the course of the study period Since however they were collected in a prospective manner for cases and controls born at the same time a bias has probably been avoided In Table 6 there are significantly more cases than controls in the poorer dwellings There was no sex difference and no change in the pattern from 1956–59 to 1968–71

Home standard

The economy of the home (Table 7) was estimated by the health visitors as being above average average or below average This was not particularly on the basis of the actual earnings by the family but according to the way in which these earnings were spent for instance whether the family could cover expenses beyond bare necessities There was a significantly larger number of cases than of controls in the below average group Of all the social variables studies the economy of the home was that which had the strongest association with SIDS This association remained unchanged throughout the study period

Table 8 lists the infant health visitor s estimate as to the mother s so called mental and physical capacity The *mental capacity* which was significantly poorer for cases than for controls, was estimated on the basis of the mother s attitude to the infant as well as her ability and determination to follow the guidance given by the infant health visitor When the material was divided into four year periods a significant difference was found only during that from 1956–59 (χ^2=7 73 d f =1 p=0 005) the difference between cases and controls decreasing as time went by (the excess percentage of above average control mothers over the above average case mothers being during the successive four year periods 26% 17% 12% and 4%) The mother s *physical capacity* in which there was no difference between

Table 7 *Percentage (and number) of cases and controls by economy of the home as classified by the infant health visitor*

Economy of the home	Cases	Controls
Above average	5 5 (5)	10 9 (51)
Average	69 2 (63)	84 6 (396)
Below average	25 3 (23)	4 5 (21)
χ^2 (d f)	46 24 (2)	
p value	<0 0001	
Unknown	(40)	(56)

Table 8 *Percentage (and number) of cases and controls by mother's mental and physical capacity as classified by the infant health visitor*

	Mother s mental capacity		Mother s physical capacity	
	Cases	Controls	Cases	Controls
Above average	39 6 (36)	56 0 (267)	60 8 (59)	63 2 (301)
Average	57 1 (52)	42 3 (202)	37 1 (36)	36 3 (173)
Below average	3 3 (3)	1 7 (8)	2 1 (2)	0 4 (2)
χ^2 (d f)	7 63 (1)		0 11 (1)	
p value	0 0058		0 74	
Unknown	(40)	(47)	(34)	(48)

cases and controls was estimated according to whether the mother had sufficient strength to look after her home and child/children

Infant care

The care of the infant including cleanliness was also estimated by the infant health visitors and classified as above average average and below average In cases where it was classified as being below average there were serious objections Table 9 shows significantly better care of controls than of cases This difference was also reduced as time went by Regrettably the criterion for classifying above average has altered in the course of the study period but an impression of the trend is gained by using $\sqrt{(\chi^2/n)}$ as a measure of case control difference the findings for the successive four year periods were 0 24 0 27 0 13 and 0 09

The percentage of *appointments not kept* (Table 10) shows that the case parents were less conscientious than the control parents in keeping the appointments In this respect there were no definite changes in the trend through the years

Malformations and morbidity

Four cases and six controls had *congenital malformations* Within the SIDS group there was one case of syndactyly one of polydactyly one of a deformed foot and bilateral testicular hydrocele and lastly one case of intestinal eventration and malrotation The malformations in the controls were foot deformities in four harelip and cleft palate in one and ureteric stricture in one Apart from actual malformations two SIDS infants had *hernia* one umbilical and one inguinal Within the control group one infant had an umbilical hernia Lastly it should be mentioned that among the SIDS cases *neonatal asphyxia* had been recorded by the midwife in three two of whom were premature (birth weights 1 400 and

Table 9 *Percentage (and number) of cases and controls by infant care as classified by the infant health visitor*

Infant care	Cases	Controls
Above average	54 3 (63)	69 8 (359)
Average	44 0 (51)	30 2 (155)
Below average	1 7 ( )	(0)
χ^2 (d f)	9 64 (1)	
p value	0 0019	
Unknown	(15)	(10)

Table 10 *Percentage (and number) of cases and controls by mother s keeping appointments with infant health visitor*

Appointments not kept (ANK)	Cases	Controls
(ANK)≤10%	55 1 (70)	72 9 (380)
10%<(ANK)≤20%	18 1 (23)	15 7 (82)
20%<(ANK)	26 8 (34)	11 3 (59)
χ^2 (d f)	22 09 (2)	
p value	0 0001	

Table 11 *Percentage (and rate) of cases and controls hospitalized for their own disease during their lifetime and within the first year of life respectively*

Also subdivided according to birth weight For 29 cases and 100 controls it is unknown whether they have been hospitalized

	All infants	Matures (birth weight >2 500 g)	Prematures (birth weight ≤2 500 g)
Cases	33 3 (=34/102)	21 8 (=17/78)	70 8 (=17/24)
Controls	*13 0 (=55/424)*	*12 9 (=53/411)*	*15 4 (=2/13)*
χ^2 (d f)	22 82 (1)	3 54 (1)	8 28 (1)
p value	<0 0001	0 060	0 004

1 500 g) In the control group there had been no instances of asphyxia

To gain an impression of the morbidity of the SIDS infants we analysed from the health visitors records how many cases had been *in hospital* because of a disease of their own (not their mother s) (Table 11) If more than 30 days had elapsed between the last visit by the infant health visitor and the infant s death and the infant had not previously been admitted to hospital, it was considered unknown whether the infant had been in hospital By way of comparison we used the number of controls who had been in hospital during the first year of life Although the average life span of the cases was only 133 days and the observation period for the controls was 365 days Table 11 shows that significantly more cases than controls had been admitted to hospital This tendency applied to matures as well as prematures but it will be noted that the greater part of the difference is explained by admissions for prematurity (*vide infra*)

In the attempt to assess the gravity of the hospital admissions we analysed for those admitted (34 cases and 55 controls) the *number of days in hospital* for each infant For matures the mean stay was 26 9 and 23 2 days for cases and controls respectively For prematures the corresponding values were 51 3 and 42 0 days Thus the duration of the stay in hospital was about 20% longer for cases but this difference is not significant judging by the Mann Whitney test

Table 12 *Percentage (and number) of 139 SIDS cases without or with symptoms or illness during the fourth to second week before death the seventh to second day before death and the last day before death*

During the period fourth to second week before death two SIDS cases had had two symptoms from the seventh to second day before death four had had two symptoms and on the last day three had had two symptoms

	Fourth to second week before death	Seventh to second day before death	Last day before death
No symptoms or illness	72 3 (81)	49 2 (62)	47 2 (59)
Respiratory symptoms or diseases	16 1 (18)	34 1 (43)	35 2 (44)
Otitis media	3 6 (4)	6 3 (8)	4 0 (5)
Gastrointestinal symptoms or diseases	4 5 (5)	10 3 (13)	8 8 (11)
Other symptoms or diseases	5 4 (6)	3 2 (4)	7 2 (9)
Unknown	(27)	(13)	(14)

Table 13 *Percentage (and number) of 139 SIDS cases grouped according to month of death*

For comparison a series of 51 infants who died of respiratory infections at the age of one week to one year have been similarly grouped (cf text) The statistical tests indicate non-uniform distributions over the year

	SIDS	Respiratory deaths
Cold months (DJFM)	42 4 (59)	49 0 (25)
Warm months (JJAS)	25 2 (35)	9 8 (5)
Remaining months (AM+ON)	32 4 (45)	41 2 (21)
χ^2 (d f)	6 27 (2)	13 18 (2)
p value	0 043	0 0014

The main *hospital diagnoses* were prematurity (13 cases 1 control) otitis media (2 cases 7 controls) respiratory diseases including pneumonia bronchitis acute laryngitis influenza and whooping cough (2 cases 10 controls) gastrointestinal conditions in particular dyspepsia and acute gastroenteritis (7 cases 14 controls) and other diagnoses (7 cases 20 controls) Lastly the diagnosis in 3 cases and 3 controls is unknown

From the police reports we have extracted data concerning all 139 SIDS cases as regards *symptoms or diseases antecedent to death* (Table 12) During the last week rather more than half the SIDS infants had had symptoms about 75% of which were respiratory symptoms or otitis media On the other hand less than 30% had had symptoms during the second to fourth week before death

The *month of death* Table 13 is grouped into the four warmest the four coldest and the four remaining months of the year A significantly larger number of SIDS cases died during the cold months From the study period 1956 to 1971 an analysis was made of deaths from respiratory infections without preceding illness at the age of one week to one year in this category too deaths occurred significantly more often during the cold months (for further description of this material cf (10)) With respect to month at death there was no sex difference within the groups Among the 131 SIDS cases for whom the health visitor s record includes information about birth weight there was also no difference in this respect between matures and prematures

DISCUSSION

Socioeconomic factors

Like most previous studies (1 2 4–6 8 9 12–15 17 18) ours has shown that the SIDS infants were from an underprivileged environment a large number being born out of wedlock and living only with their mothers having parents with not so secure occupations living in the poorer parts of town in more crowded and poor quality dwellings (Tables 1–6)

In addition the data collected by the infant health visitors gave an impression of the home standard The parents of the SIDS cases were not particularly skilled in dealing with their earnings (Table 7) and the mothers mental capacity was below that in the control group (Table 8) An estimate of maternal intelligence and effectiveness has been published previously (16) but in a broader group of infant deaths The results pointed into the same direction We also found that infant care (Table 9) was of lower standard and that the health visitors more often visited the SIDS families in vain finding no one at home at the appointed time (Table 10)

On the other hand there was no difference between the case and control groups with regard to the mothers physical capacity (Table 8) This we interpret to the effect that the psychosocial family background—indirectly reflected by the variables just mentioned—is more likely to trigger the occurrence of SIDS in the family than is insufficient maternal strength for the purely physical care of the baby At the same time it must be pointed out that the difference between cases and controls with respect to maternal mental capacity and the infant s care seems to have decreased during recent years

Table 11 *Percentage (and rate) of cases and controls hospitalized for their own disease during their lifetime and within the first year of life respectively*

Also subdivided according to birth weight For 29 cases and 100 controls it is unknown whether they have been hospitalized

	All infants	Matures (birth weight >2 500 g)	Prematures (birth weight ≤2 500 g)
Cases	33 3 (=34/102)	21 8 (=17/78)	70 8 (=17/24)
Controls	13 0 (=55/424)	12 9 (=53/411)	15 4 (=2/13)
χ^2 (d f)	22 82 (1)	3 54 (1)	8 28 (1)
p value	<0 0001	0 060	0 004

1 500 g) *In the control group there had been* no instances of asphyxia

To gain an impression of the morbidity of the SIDS infants, we analysed from the health visitors records how many cases had been *in hospital* because of a disease of their own (not their mother s) (Table 11) If more than 30 days had elapsed between the last visit by the infant health visitor and the infant s death and the infant had not previously been admitted to hospital it was considered unknown whether the infant had been in hospital By way of comparison we used the number of controls who had been in hospital during the first year of life Although the average life span of the cases was only 133 days and the observation period for the controls was 365 days Table 11 shows that significantly more cases than controls had been admitted to hospital This tendency applied to matures as well as prematures but it will be noted that the greater part of the difference is explained by admissions for prematurity (*vide infra*)

In the attempt to assess the gravity of the hospital admissions we analysed for those admitted (34 cases and 55 controls) the *number of days in hospital* for each infant For matures the mean stay was 26 9 and 23 2 days for cases and controls respectively For prematures the corresponding values were 51 3 and 42 0 days Thus the duration of the stay in hospital was about 20% longer for cases but this difference is not significant judging by the Mann Whitney test

Table 12 *Percentage (and number) of 139 SIDS cases without or with symptoms or illness during the fourth to second week before death the seventh to second day before death and the last day before death*

During the period fourth to second week before death two SIDS cases had had two symptoms from the seventh to second day before death four had had two symptoms and on the last day three had had two symptoms

	Fourth to second week before death	Seventh to second day before death	Last day before death
No symptoms or illness	72 3 (81)	49 2 (62)	47 2 (59)
Respiratory symptoms or diseases	16 1 (18)	34 1 (43)	35 2 (44)
Otitis media	3 6 (4)	6 3 (8)	4 0 (5)
Gastrointestinal symptoms or diseases	4 5 (5)	10 3 (13)	8 8 (11)
Other symptoms or diseases	5 4 (6)	3 2 (4)	7 2 (9)
Unknown	(27)	(13)	(14)

Table 13 *Percentage (and number) of 139 SIDS cases grouped according to month of death*

For comparison a series of 51 infants who died of respiratory infections at the age of one week to one year have been similarly grouped (cf text) The statistical tests indicate non uniform distributions over the year

	SIDS	Respiratory deaths
Cold months (DJFM)	42 4 (59)	49 0 (25)
Warm months (JJAS)	25 2 (35)	9 8 (5)
Remaining months (AM+ON)	32 4 (45)	41 2 (21)
χ^2 (*d f*)	6 7 (2)	13 18 (2)
p value	0 043	0 0014

The main *hospital diagnoses* were prematurity (13 cases 1 control) otitis media (2 cases 7 controls) respiratory diseases including pneumonia bronchitis acute laryngitis influenza and whooping cough (2 cases 10 controls) gastrointestinal conditions in particular dyspepsia and acute gastroenteritis (7 cases 14 controls) and other diagnoses (7 cases 20 controls) Lastly the diagnosis in 3 cases and 3 controls is unknown

From the police reports we have extracted data concerning all 139 SIDS cases as regards *symptoms or diseases antecedent to death* (Table 12) During the last week rather more than half the SIDS infants had had symptoms about 75% of which were respiratory symptoms or otitis media On the other hand less than 30% had had symptoms during the second to fourth week before death

The *month of death* Table 13 is grouped into the four warmest the four coldest and the four remaining months of the year A significantly larger number of SIDS cases died during the cold months From the study period 1956 to 1971 an analysis was made of deaths from respiratory infections without preceding illness at the age of one week to one year in this category too deaths occurred significantly more often during the cold months (for further description of this material cf (10)) With respect to month at death there was no sex difference within the groups Among the 131 SIDS cases for whom the health visitor s record includes information about birth weight there was also no difference in this respect between matures and prematures

DISCUSSION

Socioeconomic factors

Like most previous studies (1 2 4–6 8 9 12–15 17 18) ours has shown that the SIDS infants were from an underprivileged environment a large number being born out of wedlock and living only with their mothers having parents with not so secure occupations living in the poorer parts of town in more crowded and poor quality dwellings (Tables 1–6)

In addition the data collected by the infant health visitors gave an impression of the home standard The parents of the SIDS cases were not particularly skilled in dealing with their earnings (Table 7) and the mothers mental capacity was below that in the control group (Table 8) An estimate of maternal intelligence and effectiveness has been published previously (16) but in a broader group of infant deaths The results pointed into the same direction We also found that infant care (Table 9) was of lower standard and that the health visitors more often visited the SIDS families in vain *finding no one at home* at the appointed time (Table 10)

On the other hand there was no difference between the case and control groups with regard to the mothers physical capacity (Table 8) This we interpret to the effect that the psychosocial family background—indirectly reflected by the variables just mentioned—is more likely to trigger the occurrence of SIDS in the family than is insufficient maternal strength for the purely physical care of the baby At the same time it must be pointed out that the difference between cases and controls with respect to maternal mental capacity and the infant s care seems to have decreased during recent years

This tendency towards obliterating differences in the social parameters including the father s occupation, will be further elucidated in a subsequent paper (10)

It may be emphasized that to this general impression of a poorer social environment in the SIDS group there are several exceptions, in our material too viz infants from environments which are generally considered among the best

Morbidity

Like Fedrick (7) we found more malformations in the SIDS group than among the controls We also found as did Naeye et al (15) more cases of asphyxia which can of course be related to the lower average birth weight in the SIDS group All considered, it indicates a less favourable start in life However the values are too small to form the basis of definite conclusions

Evaluating the dead infants morbidity through life is very difficult as it is hard to find a relevant control group To this end we chose to analyse the number of infants who had been admitted to hospital (Table 11) and the duration of the stay in hospital For want of a better basis of comparison we compared the SIDS infants until their death with the controls during their first year of life And even so we could demonstrate a tendency to more hospital admissions and a longer duration of stay in hospital for the SIDS cases the matures as well as the prematures This must be taken to mean that the infants in the SIDS group were more and more seriously ill than the infants of the control group or at least were thought to be more in need of being admitted to hospital and of being kept there longer e g for social reasons

Froggatt et al (8) and Fedrick (7) have also compared the morbidity of SIDS cases with that in a control group Like us they found more hospital admissions among the SIDS infants than among the controls In two materials without a control group (2 4) the frequency of admissions for SIDS cases has been reported to be only 6 5% (11/170) and 10 9% (14/128) The former authors even state that the SIDS infants tended to be remarkably healthy

As regards the causes of admissions to hospital our material like that of others is too small to form the basis of conclusions However it is worth mentioning that as compared with the findings of Froggatt et al (8) and of Fedrick (7) respiratory disorders made up only a few of the admission diagnoses among our SIDS cases and corresponded to those in the controls during the first year of life

With respect to symptoms during the weeks prior to death there is also no good control group It may be expected (6) that when questioned about an infant who has suddenly died without any signs of serious disease the parents will try to attribute a certain importance even to the slightest symptoms On the other hand the parents of living controls or of controls who have died of a known cause are more apt to overlook symptoms the infant has recently had In evaluating the accumulation of symptoms among the cases during the last weeks before death (Table 12) over reporting of recent and under reporting of somewhat earlier symptoms may play a role

That respiratory symptoms are the most common ones immediately before death (Table 12) is in close accordance with the previous findings (2 4 6) that between 44 and 68% of the SIDS cases had had respiratory disorders within the last two weeks before death But the great majority of these had been colds or sniffles This corresponds to the larger number of SIDS deaths during the cold months of the year (Table 13) (2 4 7–9) in which there is also an accumulation of deaths from respiratory infections Actually the distribution of the SIDS group looks like a less accentuated version of that of respiratory deaths This is what might be expected if part of the SIDS deaths were in fact undetectable fatal respiratory infections

CONCLUSION

Our results show that as a whole the SIDS infants were from socioeconomically under privileged environments and were more often ill than their controls At the same time how ever we have observed that the association between SIDS and certain of the less favour able social parameters has disappeared during recent years It is important therefore to in vestigate in future studies whether the social differences between cases and controls are decreasing and whether other differences have taken over In a subsequent paper (10) this aspect will be studied more closely

Neither our studies nor previous ones have demonstrated a distinct pattern let alone specific causes of SIDS deaths Therefore it must be assumed that several underlying conditions exist Maybe in predisposed in fants various factors can trigger death an apparently trivial infection being one such possible factor (Tables 12 and 13) This ac cords entirely with the hypothesis that the SIDS age range represents a period of en hanced physiological vulnerability (8)

ACKNOWLEDGEMENTS

Thanks are due to the Copenhagen Police the University Institute of Forensic Medicine Copenhagen and the Municipal Agency of Infant Health Visitors Copen hagen for access to their records and to the Statistical Office of the City of Copenhagen for processing parts of the material by computer The study was aided by a grant from the Danish Medical Research Council (J No 537-739)

REFERENCES

1 Beckwith J B The sudden infant death syndrome *Curr Pr bl Ped atr* 3 3 1973

Bergman A B Ray C G Pomeroy M A Wahl P W & Beckwith J B Studies of the sud den infant death syndrome in King County Washing ton III Epidemiology *Pediatrics* 49 860 1972

3 Biering Sorensen F Jorgensen T & Hilden J Sudden infant death in Copenhagen 1956-1971 I Infant feed ng *Acta Paed at Scand* 67 129 1978

4 Borhani N O Rooney P A & Kraus J F Post neonatal sudden unexplained death in a California community *Calif Med* 118 12 1973

5 Cameron A H & Asher P Cot deaths in Bir mingham 1958-61 *Med Sci Law* 5 187 1965

6 Carpenter R G & Shaddick C W Role of in fe tion suffocation and bottle feeding in cot death An analysis of some factors in the histories of 110 cases and their controls *Br J Prev Soc Med* 19 1 1965

7 Fedrick J Sudden unexpected death in infants in the Oxford Record Linkage Area Details of preg nancy delivery and abnormality in the infant *Br J Prev Soc Med* 28 164 1974

8 Froggatt P Lynas M A & MacKenzie G Epi demiology of sudden unexpected death in infants (cot death) in Northern Ireland *Br J Prev Soc Med* 25 119 1971

9 Houstek J Sudden infant death syndrome in Czechoslovakia Epidemiologic aspects *In* A B Bergman J B Beckwith & C G Ray (eds) *Sudden infant death syndrome* University of Wash ington Press Seattle and London 1970 p 55

10 Jørgensen T Biering Sørensen F & Hilden J Sudden infant death in Copenhagen 1956-1971 III Perinatal and perimortal factors *Acta Paediatr Scand* 68 11 1979

11 Jørgensen T Biering Sørensen F & Hilden J Sudden infant death in Copenhagen 1956-1971 IV Infant development To be published

12 Kraus A S Steele R Thompson M G & de Grosbois P Further epidemiologic observations on sudden unexpected death in infancy in Ontario *Can J Public Health* 62 210 1971

13 Kraus J F & Borhani N O Post neonatal sudden unexplained death in California A cohort study *Am J Epidemiol* 95 497 1972

14 McWeeny P M & Emery J L Unexpected post neonatal deaths (cot deaths) due to recognizable dis ease *Arch Dis Child* 50 191 1975

15 Naeye R L Ladis B & Drage J S Sudden infant death syndrome A prospective study *Am J Dis Child* 130 1207 1976

16 Richards I D G & McIntosh H T Confidential inquiry into 226 consecutive infant deaths *Arch Dis Child* 47 697 1972

17 Valdes Dapena M A Sudden and unexpected death in infancy A review of the world literature 1954-1966 *Pediatrics* 39 123 1967

18 Valdes Dapena M Birle L J McGovern J A McGillen J F & Colwell F H Sudden unexpected death in infancy A statistical analysis of certain socioeconomic factors *J Pediatr* 73 387 1968

Submitted March 11 1978
Accepted May 19 1978

(J H) Institute of Human Genetics
University of Copenhagen
Rådmandsgade 71 A
DK 2200 Copenhagen N
Denmark

This tendency towards obliterating differences in the social parameters including the father s occupation will be further elucidated in a subsequent paper (10)

It may be emphasized that to this general impression of a poorer social environment in the SIDS group there are several exceptions in our material too viz infants from environments which are generally considered among the best

Morbidity

Like Fedrick (7), we found more malformations in the SIDS group than among the controls We also found as did Naeye et al (15) more cases of asphyxia which can of course be related to the lower average birth weight in the SIDS group All considered it indicates a less favourable start in life However the values are too small to form the basis of definite conclusions

Evaluating the dead infants morbidity through life is very difficult as it is hard to find a relevant control group To this end we chose to analyse the number of infants who had been admitted to hospital (Table 11) and the duration of the stay in hospital For want of a better basis of comparison we compared the SIDS infants until their death with the controls during their first year of life And even so we could demonstrate a tendency to more hospital admissions and a longer duration of stay in hospital for the SIDS cases the matures as well as the prematures This must be taken to mean that the infants in the SIDS group were more and more seriously ill than the infants of the control group or at least were thought to be more in need of being admitted to hospital and of being kept there longer e g for social reasons

Froggatt et al (8) and Fedrick (7) have also compared the morbidity of SIDS cases with that in a control group Like us they found more hospital admissions among the SIDS infants than among the controls In two materials without a control group (2 4) the frequency of admissions for SIDS cases has been reported to be only 6 5% (11/170) and 10 9% (14/128) The former authors even state that the SIDS infants tended to be remarkably healthy

As regards the causes of admissions to hospital our material like that of others is too small to form the basis of conclusions However it is worth mentioning that as compared with the findings of Froggatt et al (8) and of Fedrick (7) respiratory disorders made up only a few of the admission diagnoses among our SIDS cases and corresponded to those in the controls during the first year of life

With respect to symptoms during the weeks prior to death there is also no good control group It may be expected (6) that when questioned about an infant who has suddenly died without any signs of serious disease the parents will try to attribute a certain importance even to the slightest symptoms On the other hand the parents of living controls or of controls who have died of a known cause are more apt to overlook symptoms the infant has recently had In evaluating the accumulation of symptoms among the cases during the last weeks before death (Table 12) over reporting of recent and under reporting of somewhat earlier symptoms may play a role

That respiratory symptoms are the most common ones immediately before death (Table 12) is in close accordance with the previous findings (2 4 6) that between 44 and 68% of the SIDS cases had had respiratory disorders within the last two weeks before death But the great majority of these had been colds or sniffles This corresponds to the larger number of SIDS deaths during the cold months of the year (Table 13) (2 4 7–9) in which there is also an accumulation of deaths from respiratory infections Actually the distribution of the SIDS group looks like a less accentuated version of that of respiratory deaths This is what might be expected if part of the SIDS deaths were in fact undetectable fatal respiratory infections

Acta Pædiatr Scand 68 11–22 1979

SUDDEN INFANT DEATH IN COPENHAGEN 1956–1971

III *Perinatal and Perimortal Factors*

TORBEN JØRGENSEN FIN BIERING SØRENSEN and JØRGEN HILDEN

From the Municipal Agency of Infant Health Visitors Copenhagen and the Institute of Human Genetics University of Copenhagen Copenhagen Denmark

ABSTRACT Jørgensen T Biering Sørensen F and Hilden J (Municipal Agency of Infant Health Visitors Copenhagen and Institute of Human Genetics University of Copenhagen Copenhagen Denmark) Sudden infant death in Copenhagen 1956–1971 III Perinatal and perimortal factors Acta Paediatr Scand 68 11 1979.—139 cases of the Sudden Infant Death Syndrome (SIDS) among infants born in the Municipality of Copenhagen during the period 1956–1971 were analysed on the basis of data collected from police reports and (for 131 cases) from the infant health visitors records In the SIDS group there was a greater male preponderance than among others dying in the same age range Compared with the living controls the SIDS mothers had attended less prenatal examinations more often delivered their babies at home the SIDS parents were younger and yet the SIDS infants were less often firstborns There was no difference with respect to history of abortions maternal state of health during pregnancy or events at delivery The age at death for the SIDS infants is of a distribution similar to that for fatal respiratory infections Prematures died later than matures but this difference is not statistically significant It is concluded that perinatal factors and SIDS are correlated but owing to changes in predisposing factors and decreasing differences between cases and controls in recent years as well as interdependence of the factors it seems doubtful whether the incidence of SIDS can be reduced by alleviating the above mentioned unfavourable factors

KEY WORDS Sudden infant death social conditions prenatal care abortions birth order respiratory tract infections

The Sudden Infant Death Syndrome (SIDS) and its correlation to various perinatal and perimortal factors have been elucidated in several previous studies (2 8 9 17 18 22 25) After the theory of external suffocation as a cause of SIDS has been gradually abandoned in the middle of this century (2 25) the conditions under which the infant is found dead have not attracted major interest Instead the age at death and the numerous factors relating to gestation and labour have given food for further research and theorizing (2 8 9 18 24 27)

On the basis of data collected prospectively by the infant health visitors police report data and extracts of death certificates from the National Health Service we have tried by the present study to gain further and more detailed information about these factors It was endeavoured also to demonstrate the interdependence of these factors and their change in time parallel with the findings in a previous study (6) In the light of our findings the possibilities of reducing the incidence of SIDS are discussed

The material forms part of a larger epidemiological study of SIDS in the Municipality of Copenhagen (5 6 13)

MATERIAL AND METHODS

For the delimitation of the material we adopted the following modification of the SIDS definition proposed by Beckwith at the Second International Conference on the Causes of SIDS in 1969 (3) The sudden death at home of infants between one week and one year which is unexpected by history and in which a thorough post mortem examination fails to demonstrate an adequate cause of death

Table 2. *Percentage and (number) of cases and controls born at home, in maternity clinics and in hospitals*

Total divided into birth order one and more than one

Place of birth	Total		Birth order one		Birth order more than one	
	Cases	Controls	Cases	Controls	Cases	Controls
Home	21 (26)	10 (47)	5 (2)	4 (8)	27 (23)	15 (39)
Maternity clinic	19 (24)	27 (127)	13 (5)	27 (56)	22 (19)	27 (71)
Hospital	60 (75)	63 (301)	82 (31)	69 (145)	51 (44)	59 (156)
χ^2 (d.f.)	12.12 (2)		3.79 ( )		6.66 (2)	
p-value	0.0022		0.19		0.036	
Unknown	(6)	(49)	(5)	(28)	(1)	(21)

A few studies (16, 25) have shown a female and the remainder (2, 7, 20, 22, 25) a male SIDS preponderance. Froggatt et al. (11) found 58.8% boys in the SIDS group against 53.2% among all other deaths within the same age range.

Prenatal care

In Denmark nine prenatal *visits to the doctor and/or midwife* are recommended. We consider eight or more visits to be satisfactory attendance.

As seen from Table I, a significantly larger number of SIDS than control mothers did not give satisfactory attendance, and this is further illustrated by the mean number of visits. A particularly large number of SIDS mothers paid only 1–3 visits. There was no sex difference.

Division of the material. Division by place of birth shows the same difference as described above for mothers having their babies in hospital or maternity clinics. Only mothers having their babies at home show no significant difference in attendance (Table 1). Thus what constitutes the major part of the difference in the Total portion of the table is the large number of control mothers with satisfactory prenatal attendance and having their babies in hospitals or maternity clinics.

We also divided the material by *birth order*, viz. firstborns and others, but this showed no difference from the pattern in the Total of Table 1. There was, not unexpectedly, a tendency to fewer prenatal visits at a high birth order among case as well as control mothers.

Maternal health during pregnancy and course of delivery

In 19 (15%) of the SIDS mothers and 70 (14%) of the control mothers the *state of maternal health during pregnancy* was not considered good. The disorders in question were preeclampsia, nausea and vomiting (emesis), anaemia and various trivial diseases such as mild infections. As regards mild infections, there were five incidents among the 70 control mothers but none among the 19 SIDS mothers.

In 9 (7%) of the SIDS mothers and 27 (5%) of the control mothers the *delivery* had not been normal. Either it had been operative—by forceps, vacuum extractor or caesarean section—or else the foetus had presented by the breech. There was no sex difference.

Place of birth

A distinction will be made between three places of birth, viz. *at home*, in a *maternity clinic* and in *hospital*. In Denmark a maternity clinic is a maternity institution having certain technical facilities and a fixed staff of midwives. A doctor is not constantly present, but

Table 1 *Percentage and (number) of case and control mothers visiting the doctor and/or mid wife less than eight times for prenatal care*

Total divided into deliveries at home and in maternity institutions For 11 cases and 28 controls attendance was unknown

No of visits	Total		Born at home		Born in maternity clinic/hospital	
	Cases	Controls	Cases	Controls	Cases	Controls
<8	48 (57)	33 (164)	44 (11)	43 (19)	48 (46)	32 (145)
≥8	52 (63)	67 (332)	56 (14)	57 (25)	52 (49)	68 (307)
χ^2 (d f)	8 14 (1)		0 034 (1)		8 52 (1)	
p value	0 0044		0 85		0 0035	
Mean	7 04	8 33	7 00	7 57	7 05	8 41

A further description of the selection was given in the first paper of the present series (5)

Our material comprises all 139 SIDS cases born in the Municipality of Copenhagen during the period 1956 to 1971 For all cases we have information from the police reports which are mandatory in such cases Furthermore the prospectively collected data from the municipal infant health visitors records were available for 131 of the cases (44 girls and 87 boys) (5)

Each of these 131 SIDS cases was matched with four controls from the infant health visitors files These con trols were living at home and with a few exceptions they were of the same sex and born on the same day (5)

From the National Health Service we obtained informa tion about the sex age at death and cause of death for all infants born in the Municipality of Copenhagen during the period 1956–1971 and dying at the age of one week to one year

Data concerning perimortal factors such as circum stances at death and the time when found dead are from the police reports whereas the perinatal data are derived from the health visitors records Let it be mentioned that with the exception of maternal health during pregnancy the perinatal data are also included in the midwife s re cord sent to the infant health visitor prior to her first visit to the home

It must be pointed out that the so called perinatal fac tors to be elucidated here are often of a social nature and their distinction from the social factors previously re ported (6) is therefore arbitrary Indeed this will be ap parent from our conclusions

In assessing time trends we shall refer unless otherwise stated to the following four year periods 1956–59 1960–63 1964–67 and 1968–71 which are further characterized in the preceding paper (6)

The statistical tests used were the chi square Mann Whitney rank sum test and combinatorial reasoning Sig nificance level 5%

RESULTS

Incidence

The incidence of SIDS was 0 92 cases per 1 000 live born as stated in the first paper of this series (5) At the same time it was pointed out that the incidence had remained constant through the period 1956 to 1971 while among whites in San Francisco (26) the incidence had fallen from 1 2 in 1961 to 0 7 in 1971

Within the material of 131 SIDS cases there were 26 (19 8%) prematures (birth weight ≤2 500 g) and among the 524 controls 16 (3 1%)

During the time under study 1956–71 the SIDS deaths made up 15 7% of all deaths in the age range concerned This proportion has not been constant the SIDS cases amounting to 12 6% (66/525) during the years 1956–63 and to 20 3% (73/360) during the years 1964–71 This significant change (χ^2=9 01 d f =1 p= 0 0027) reflects a fall in general infant mortal ity while the incidence of SIDS remained con stant In two previously published studies the SIDS cases have made up about one third of the deaths occurring from 1 week to 1 year (2) and from 4 weeks to 51 weeks (12)

Sex ratio

In the material of 139 SIDS cases 94 (68%) were boys and 45 (32%) girls Among the re maining infants born in the Municipality of Copenhagen from 1956 to 1971 and dying at the age of one week to one year 419 (56%) were boys and 327 (44%) girls The difference in the sex ratio between the SIDS cases and the others is statistically significant (χ^2=5 85 d f =1 p=0 016)

Table 4 *Percentage and (number) of case and control mothers whose age at delivery was ≤19 20–24 25–29 and ≥30 and whose civil status was unmarried and married*

	Total		Mother unmarried		Mother married	
Age	Cases	Controls	Cases	Controls	Cases	Controls
≤19	18 (23)	7 (35)	?9 (9)	17 (6)	14 (14)	6 (?9)
0–?4	56 (73)	43 (?25)	58 (18)	46 (16)	56 (55)	43 (?09)
25–?9	18 (?4)	31 (159)	6 (?)	23 (8)	2? (?2)	31 (151)
≥30	8 (10)	19 (100)	6 (?)	14 (5)	8 (8)	?0 (95)
χ^2 (d f)	31 39 (3)		5 3? (2)		18 66 (3)	
p value	<0 0005		0 070		<0 0005	
Mean age	?? 8	25 5	21 9	23 9	23 1	25 6
Unknown	(1)	(5)	(0)	(1)	(1)	(4)

week) are included but not previous abortions

According to Table 6 the SIDS cases were more rarely firstborns than the controls There was no sex difference

Division of the material Matures and prematures showed the same tendency

The pattern has altered in the course of time (Table 7) In 1956–59 there was a marked preponderance of SIDS cases of a birth order of two or more but in 1968–71 this feature had vanished

Abortions

Abortion is defined here as provoked or spontaneous interruption of a pregnancy before the 28th week producing a dead foetus

None of the women had a history of more than four abortions There was no significant case control difference (Table 8) No sex difference

Among the mothers in Table 8 with a history of abortions there is a tendency to multiple (i e 2–4) abortions in SIDS mothers However the difference is hardly significant (n= 114 χ^2=3 83 d f =1 p=0 050)

Lastly we compared the total number of abortions with the total number of pregnancies This revealed (Table 8) a high abortion rate in SIDS mothers but the difference is not significant (n=1 142 pregnancies χ^2=0 17 d f =1 p=0 68)

Maternal age corrected for parity

The pattern showing a higher birth order for cases than for controls (Table 6) is seen also when the material is divided by maternal age

Table 5 *Percentage and (number) of case and control fathers whose age at the birth of the children was ≤24 25–29 30–34 and ≥35*

Father s age	Cases	Controls
≤ 4	45 (45)	8 (137)
?5–?9	31 (31)	37 (180)
30–34	1 (1)	?0 (98)
≥35	1 (1)	14 (69)
χ^2 (d f)	11 5 (3)	
p-value	0 005<p<0 010	
Mean age	6 8	28 5
Unknown	(31)	(40)

Table 6 *Percentage and (number) of cases and controls divided by birth order one two and three or more*

Birth order	Cases	Controls
1	33 (43)	45 (237)
?	4? (54)	36 (187)
≥3	25 (33)	19 (100)
χ^2 (d f)	6 64 (2)	
p-value	0 036	
Unknown	(1)	(0)

Table 3 *Percentage and (number) of cases and controls born at home in maternity clinics and in hospitals for four periods of time*

Cases tested against controls for the periods 1956–63 and 1964–71 since otherwise the numbers would be too small

Place of birth	1956–59		1960–63		1964–67		1968–71	
	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
Home	29 (9)	20 (24)	32 (9)	7 (7)	18 (7)	5 (7)	4 (1)	9 (9)
Maternity clinic	19 (6)	28 (33)	14 (4)	25 (25)	24 (9)	31 (47)	18 (5)	21 (22)
Hospital	52 (16)	53 (63)	54 (15)	68 (67)	58 (22)	65 (99)	79 (22)	70 (72)
χ^2 (d f)		9 14(2)				3 13 (2)		
p value		*0 010*				*0 21*		

a general practitioner or an obstetrician is routinely called so that only a few deliveries take place without a doctor being present

Table 2 shows that a significantly larger proportion of cases than of controls were born at home while there was a preponderance of controls born in maternity clinics With respect to deliveries in hospital there was no major difference There was no sex difference

Division of the material Considering firstborns and others (Table 2) the latter group follows the pattern from the Total of the table Within the group of firstborns there were only a few births at home and in this respect there was no difference between cases and controls At the same time there was a preponderance of hospital births for cases

If the married and unmarried mothers are considered separately the married ones follow the pattern from the Total in Table 2 whereas—not surprisingly—the unmarried mothers follow the pattern of the primiparae Nearly all unmarried mothers were delivered in hospital (cases 94%, controls 81%)

A division into matures and prematures shows for both groups the same pattern as in the Total of Table 2

In Table 3 the material is divided into four chronological periods It will be seen that births at home have been declining among cases as well as controls This is more striking for cases the preponderance of home deliveries in the SIDS group (Table 2) having disappeared in 1968–71 Besides the difference between the proportion of cases and controls born in maternity clinics is decreasing

The birth place distribution of the control group altered significantly with time (Table 3 $\chi^2=8\ 43$ d f $=2$ $p=0\ 015$)

Age of parents

The SIDS *mothers* were significantly younger (Table 4) the pattern being the same for married and unmarried mothers There was no sex difference

Division of the material Matures showed the same pattern (maternal mean age 22 6 years in the SIDS group ($n=105$) and 25 6 years in the control group ($n=505$)) as in the Total of Table 4 This typical pattern was not present among the prematures the maternal mean age for SIDS cases being 23 7 years ($n=25$) and that for controls 23 0 years ($n=16$)

The difference in mean age between SIDS and control mothers diminished in the course of time (1956–59 4 3 years 1960–63 2 8 years 1964–67 2 4 years and 1968–71 1 5 years)

Paternal age (Table 5) showed the same pattern as maternal age Only the fathers were about 3 years older Fathers of unknown age were mainly those who were not living with the mothers There was no age difference between the fathers of boys and girls

Parity

In calculating the *birth order* previous still births (the birth of a dead foetus after the 28th

Table 4 *Percentage and (number) of case and control mothers whose age at delivery was ≤19 20–24 25–29 and ≥30 and whose civil status was unmarried and married*

	Total		Mother unmarried		Mother married	
Age	Cases	Controls	Cases	Controls	Cases	Controls
≤19	18 (23)	7 (35)	29 (9)	17 (6)	14 (14)	6 (29)
20–24	56 (73)	43 (225)	58 (18)	46 (16)	56 (55)	43 (209)
25–29	18 (24)	31 (159)	6 (2)	23 (8)	22 (22)	31 (151)
≥30	8 (10)	19 (100)	6 (2)	14 (5)	8 (8)	20 (95)
χ^2 (d f)	31 39 (3)		5 3[illegible] ([illegible])		18 66 (3)	
p value	<0 0005		0 070		<0 0005	
Mean age	22 8	25 5	21 9	23 9	23 1	25 6
Unknown	(1)	(5)	(0)	(1)	(1)	(4)

week) are included but not previous abortions

According to Table 6 the SIDS cases were more rarely firstborns than the controls There was no sex difference

Division of the material Matures and prematures showed the same tendency

The pattern has altered in the course of time (Table 7) In 1956–59 there was a marked preponderance of SIDS cases of a birth order of two or more but in 1968–71 this feature had vanished

Abortions

Abortion is defined here as provoked or spontaneous interruption of a pregnancy before the 28th week producing a dead foetus

None of the women had a history of more than four abortions There was no significant case control difference (Table 8) No sex difference

Among the mothers in Table 8 with a history of abortions there is a tendency to multiple (i e 2–4) abortions in SIDS mothers However the difference is hardly significant (n= 114 χ^2=3 83 d f =1 p=0 050)

Lastly we compared the total number of abortions with the total number of pregnancies This revealed (Table 8) a high abortion rate in SIDS mothers but the difference is not significant (n=1 142 pregnancies χ^2=0 17 d f =1 p=0 68)

Maternal age corrected for parity

The pattern showing a higher birth order for cases than for controls (Table 6) is seen also when the material is divided by maternal age

Table 5 *Percentage and (number) of case and control fathers whose age at the birth of the children was ≤24 25–29 30–34 and ≥35*

Father s age	Cases	Controls
≤24	45 (45)	28 (137)
25–29	31 (31)	37 (180)
30–34	1[illegible] (1[illegible])	20 (98)
≥35	1[illegible] (1[illegible])	14 (69)
χ^2 (d f)	11 5 (3)	
p-value	0 005<*p*<0 010	
Mean age	[illegible]6 8	[illegible]8 5
Unknown	(31)	(40)

Table 6 *Percentage and (number) of cases and controls divided by birth order one two and three or more*

Birth order	Cases	Controls
1	33 (43)	45 (237)
2	42 (54)	36 (187)
≥3	25 (33)	19 (100)
χ^2 (d f)	6 64 (2)	
p-value	0 036	
Unknown	(1)	(0)

Table 7 *Percentage and (number) of cases and controls whose birth order was one two an three or more divided into four periods of time*

Birth order	1956–59		1960–63		1964–67		1968–71	
	Cases	*Controls*	*Cases*	*Controls*	*Cases*	*Controls*	*Cases*	*Controls*
1	13 (4)	38 (48)	31 (9)	40 (46)	38 (15)	55 (90)	48 (14)	45 (51)
2	65 (20)	43 (54)	28 (8)	32 (37)	43 (17)	31 (51)	31 (9)	38 (43)
≥3	23 (7)	19 (24)	41 (12)	28 (32)	20 (8)	14 (23)	21 (6)	18 (20)
χ^2 (d f)	7 46 (2)		2 12 (2)		3 91 (2)		0 45 (2)	
p value	0 024		0 35		0 14		0 79	

(Table 9), except in the group aged 30 and over

For each of the birth orders the SIDS mothers are younger than the control mothers (Fig 1) On the basis of this curve it is tempting to assume that the second child follows soon upon the first in SIDS mothers

By regarding the maternal mean age at the various birth orders in the control group as the age at which a woman normally has her first (second, third) baby it is possible to elucidate the effect of maternal age corrected for parity In this way the women may be divided into two groups Group A consisting of mothers who were young compared with the number of children they had borne (i e one child and less than 23 years two children and less than 26 years, three children and less than 29 years and four or more children and less than 31 years) and group B comprising the others Table 10 shows that a significant surplus of SIDS mothers were young for parity

If this principle is applied to the four perio separately (Table 11) the difference betwee SIDS cases and controls will be seen to b decreasing in time

Infant s age at death

The three curves in Fig 2 show the *age a death* (between one week and one year) fo SIDS cases for all other infants born in th Municipality of Copenhagen in 1956–71 an for those of the latter who died of respirator tract infections without pre existing chroni disease The diagnoses in the last mentione group were Acute pharyngitis acute laryngi tis and tracheitis acute bronchitis lobar pneu monia bronchopneumonia and empyema These diagnoses are according to the WH(classification

The curve for all non SIDS deaths shows a marked accumulation of deaths within the firs month and is obviously different from th

Table 8 *Percentage and (number) of cases and controls whose mothers had a history of no one and 2–4 abortions*

No of abortions	Cases	Controls
None	82 (98)	81 (401)
1	12 (14)	16 (78)
2–4	7 (8)	2 (14)
χ^2 (d f)	5 06 (2)	
p value	0 080	
Total abortions / Total pregnancies	13 4 (33/246)	12 2 (109/896)
Unknown	(11)	(31)

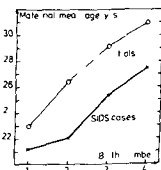


Fig 1 Mean age of SIDS and control mothers at delivery plotted against birth order (first second third fourth or more child)

Table 9 *Percentage and (number) of cases and controls of birth order one, two and three or more when maternal age at delivery was ≤19, 20–24, 25–29 and ≥30*

Birth order	≤19		20–24		25–29		≥30	
	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
1	59 (13)	91 (32)	32 (23)	59 (133)	17 (4)	36 (57)	20 (2)	13 (13)
2	41 (9)	9 (3)	51 (37)	32 (71)	17 (4)	44 (70)	40 (4)	41 (41)
≥3	0 (0)	0 (0)	18 (13)	9 (21)	66 (16)	20 (32)	40 (4)	46 (46)
χ^2 (d f)	6 67 (1)		17 04 (2)		23 35 (2)		0 0009 (1)	
p-value	0 0099		0 0002		<0 000045		0 98	

other two curves The SIDS curve has a low start in order to reach a peak at the age of 2–4 months We dare not attach importance to the fact that this peak is cleft The curve representing deaths from respiratory tract infections is rather similar to that of SIDS cases

We did not observe any sex difference in any of the three materials with respect to age at death

For the 139 SIDS cases the mean life span was 132 days 30% had died before 3 months and 76% before 6 months of age

The mean life span of infants succumbing to respiratory tract infections exceeded that of SIDS infants by only 16 days The difference was in fact not significant according to the Mann Whitney test ($t=0\ 98$ d f =188 $0\ 30<p<0\ 40$)

The mean life span of the 26 premature SIDS cases was 152 days and that of the 105 mature SIDS cases 128 days This difference is not significant according to the Mann Whitney test ($t=1\ 54$ d f =129 $0\ 10<p<0\ 20$)

Table 10 *Percentage and (number) of cases and controls whose mothers were young for parity (A) and the remainder (B)*

For further explanation see the text

	Cases	Controls
A	84 (108)	[illegible]0 (258)
B	16 (1)	[illegible]0 (61)
χ^2 (d f)	47 5 (1)	
p-value	<0 0001	

Circumstances at death

17 (13%) died while sleeping in the same bed as another person the others while lying alone in a pram carry cot cradle child s bed or adult bed 16 of the 17 who died while sharing a bed with others were boys This sex difference is significant ($n=134$ $\chi^2=4\ 59$ d f =1 $p=0\ 032$)

79 (62%) were found with vomit or frothy fluids in the mouth and nostrils commonly blood tinged staining the bedclothes In this respect there was no sex difference

41 (47%) 22 (25%) and 25 (28%) were found dead lying prone supine and on their side respectively A significantly larger number of boys were found lying supine whereas

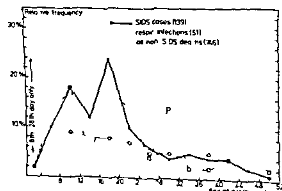


Fig 2 Curves showing the age at death for SIDS cases all other infants dying and infants dying of respiratory tract infections without preceding chronic disease All three curves represent infants born in the Municipality of Copenhagen dying during the age interval 1 week – 1 year Numbers in brackets

Table 11 *The procedure of Table 10 applied to each of the four periods of time*

	1956–59		1960–63		1964–67		1968–71	
	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
A	90 (28)	48 (60)	90 (26)	47 (54)	75 (30)	48 (78)	69 (?0)	49 (56)
B	10 (3)	52 (66)	10 (3)	53 (61)	25 (10)	52 (86)	31 (9)	51 (58)
χ^2 (d f)	16 72 (1)		15 42 (1)		8 65 (1)		2 90 (1)	
p value	<0 0001		<0 0001		0 0033		0 089	

more girls were found lying on their side (χ^2= 8 48 d f =2 p=0 014)

In 35 cases it was recorded in the police reports that the infants had been found dead their face down or covered with a pillow or eiderdown In this respect there was no sex difference

The percentages in this section were calculated on the basis of how many police reports mentioned the factor concerned

Hour when found dead

Fig 3 illustrates the *hour of the day or night* when the infant was found dead This was known for 90 boys and 40 girls There is an accumulation in the morning comprising almost all the girls and the majority of the boys In addition a small proportion of the boys were found during the afternoon or evening This sex difference is significant (p=0 022) assessed as the probability that there is a series *of 24 boys interrupted by only one girl if* 90 boys and 40 girls are arranged randomly around the clock

The interval between last seen alive and found dead averaged 4 1 hours (range 0–13 hours median 4 hours n=122) No infant was observed at the moment of death

DISCUSSION

Perinatal factors

The perinatal characteristics of which we found a preponderance in the SIDS group were Less attendance for prenatal examinations (Table 1) young parents (Tables 4 and 5) birth order two and over (Table 6) and delivery at home (Table 2) This agrees with previous studies (1 2 8 11 12 14 15, 17 18 2? 23 25) In a Canadian study (14) however i is stated that the difference in maternal age a delivery was unimpressive and not statisti cally significant (80 cases and 80 controls) but the SIDS mothers age at first delivery wa lower than that of the control mothers In a re port from Australia (1) the parity for 36 case was compared with that for all live born *during the same period and there* was littl difference No authors have discussed de liveries at home *versus* deliveries in institu tions

The increased risk of SIDS among mother who are young for parity which seems to b the natural interpretation of Table 11 support the findings of Steele & Langworth (22) viz that the SIDS mothers were younger than th control mothers when cases and controls wer matched according to parity Since moreover a high parity is an *added risk factor* at a give maternal age (Table 9) age and parity appea

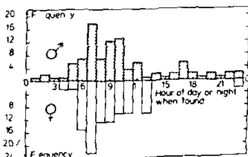


Fig 3 Hour of day or night at which the SIDS cases were found dead divided into boys (90 in all) and girls (40 in all) Midnight=0=24 hrs With one single exception no girls were found dead in the hours of the afternoon or evening

to exert an opposed influence upon the risk of SIDS

Thus SIDS mothers are characterized by earlier or more frequent pregnancies but the proportion of these pregnancies ending in abortions is not demonstrably increased

It is difficult to make comparison with other studies as regards abortions as the definitions of abortions have differed or been lacking Most authors report no difference between case and control mothers (8 11 14 21) However Naeye (17) found more abortions among SIDS mothers whereas Steele & Langworth (22) found more previous abortions among control mothers

As regards the state of health during pregnancy and the course of delivery our negative results correspond largely to previous findings (12 14 17 18) but three publications (9 17 18) have reported a preponderance of various diseases during pregnancy among SIDS mothers

For some of the parameters studied viz prenatal care (Table 1) place of birth (Table 2) maternal age (Table 4) and parity we divided the Total in the tables into various sub groups in order to be able to compare cases and controls that shared a characteristic of established importance These sub-divisions were done in the attempt to demonstrate the inter relationship of various factors predisposing to SIDS More precisely we tested how one predisposing factor acts provided that another predisposing factor is already present Summing up these sub-divisions revealed no preponderance of SIDS characterized by (*a*) a low attendance for prenatal examinations by doctor and midwife provided that the birth took place at home (Table 1) (*b*) home deliveries provided that the mother was unmarried (cf under Place of birth) or (*c*) young mothers provided that the infant was premature (cf under Age of parents) However these unfavourable groups were small On the other hand there was a preponderance of SIDS cases characterized by (*a*) delivery at home even provided that the birth order was higher than one (Table 2) (*b*) low attendance for prenatal examinations even provided that the birth order was higher than one (cf under Prenatal care) (*c*) young mothers even provided that they were unmarried (Table 4) and (*d*) high parity even provided that the infants were premature (cf under Parity) The finding that several predisposing factors are explicable by other factors supports the suspicion that a number of these perinatal factors are merely an indirect measure of unfavourable conditions and that continued research efforts might isolate a few factors of fundamental importance The multivariate statistical methods which purport to be designed to unravel this type of complicated causal relations have regrettably given imperspicuous results (2 12) possibly because of too small samples

In pointing out risk groups with a view to prevention it is important to bear in mind the lacking knowledge of the essential factors Therefore this type of prevention does not have much prospect of success at present

In the present as well as in a previous paper (6) we have tried to elucidate the behaviour of some of the predisposing factors in the course of time Among controls we have demonstrated a decrease in deliveries at home fewer infants born in wedlock (6) and a decrease in the person/room ratio (6) In other words there has been a decrease in several unfavourable factors in the population without a demonstrable decrease in the incidence of SIDS during the same period (5) By following the case control differences through time it is seen that several factors show a distance in 1956–59 but a gradual approach up to 1968–71 These factors are place of birth (Table 3) birth order (Table 7) maternal age maternal age corrected for parity (Table 11) father s occupation (6) mother s mental capacity (6) and the quality of infant care (6)

Thus some of the factors emphasized here as well as in other papers as predisposing to SIDS have undergone a change in the course of time This time trend contributes to the difficulty in defining risk groups

Age at death

In all materials the distribution by age at death (1, 2 7, 11, 15 20 22 25) has shown the same tendency viz few deaths during the first month of life a peak at two to four months followed by a rapid fall Accordingly by far the greater part of the deaths occur within the first six months, ranging in the available series from 65% to 100%

Raring (19) pooled age at death data from seven materials This gave a reasonably smooth curve which he interpreted as being log normal In his opinion this supported the theory that SIDS was a separate distinct disease entity

If SIDS is to be considered a disease entity it would be reasonable to compare it with the age at death curves for other disease groups In our Fig 2 it is very similar to the curve for deaths from respiratory tract infections This indeed agrees with the findings that (*a*) respiratory symptoms are quite common during the days preceding death and that (*b*) there is an accumulation of deaths during the cold months (6) This is supported by an official report from England (16) Richards & McIntosh (20) compared the age at death for SIDS cases with those for children dying of gastroenteritis ($n = 28$) and pneumonia ($n = 36$) without being able to find any striking resemblance The SIDS curve differs widely from that for other infant deaths considered together (Fig 2) as is indeed well known (15)

An author from New Zealand (24) like us has reported that premature SIDS cases die later than mature ones but the difference is not significant In two publications (4 10) it is merely mentioned without giving any figures that the age at death of prematures and matures does not differ significantly These reflections are of importance in assessing the theory that SIDS occurs during a vulnerable period of physiological development corresponding to the characteristic narrow age distribution at death (11 12) because if premature cases die later than mature cases this phenomenon may be interpreted to reflect that prematures take longer to reach the vulnerable period

Circumstances at death and time when found dead

That 13% died while sharing a bed with an adult supports the idea of instantaneous death without any death struggle (4) It would be of interest to compare the position when found with these and other infants normal sleeping position and the position of other dead infants This has been done but the results have not been consistent (2 4 12)

Other materials (2 4 12 23) support our finding that most of the infants are found dead in the morning or less often, in the afternoon These hours correspond to the infants dying in their sleep during the night or during the midday nap The sex difference in this respect like the other sex differences is not explicable

CONCLUSIONS

In this and in the preceding paper (6) we have confirmed that SIDS families are under privileged as regards several social factors and perinatal factors of social nature We have also pointed out that some of the factors can explain each other and we have emphasized the tendency for certain unfavourable factors not to accumulate any longer in SIDS cases and families We have quoted the theory that a physiologically vulnerable period (11 12) underlies SIDS which is suggested by the age curve and that various (unknown?) external factors need be only small to cause the infant s death if they occur during the vulnerable period In view of this theory one might think that protection against or improvement of external factors would—at least to some extent—reduce the incidence of SIDS One external factor might be for instance a trivial infection which again might be elicited by various influences of social nature—including older siblings as possible vectors

But how is the constant incidence of SIDS

(5) to be reconciled with the finding that some predisposing factors are decreasing and that the case-control difference with respect to some factors has diminished? Have other pre disposing factors become more prevalent during the same period thus maintaining *sta tus quo*? Have new factors taken over? Or is it possible that the named factors used to be re lated to the actual cause(s) but no longer are—owing to the change in social structure? Not knowing the answers we can probably not alter the incidence of SIDS by improving social and perinatal conditions or by super vising special risk groups Further information is needed and it might be of interest to ascer tain how other predisposing and also appar ently harmless factors alter in time as com pared with the incidence of SIDS

ACKNOWLEDGEMENTS

Our thanks are due to the Copenhagen Police the Uni versity Institute of Forensic Medicine Copenhagen the Municipal Agency of Infant Health Visitors Copenhagen and the National Health Service for giving us access to the material Moreover we are indebted to The Statisti cal Office of the City of Copenhagen for computer pro cessing of part of the material The study was aided by a grant from the Danish Medical Research Council (J No 55?-239)

REFERENCES

1 Beal S Sudden infant death syndrome *Med J Aust* 2 1 3 197?

Beckwith J B The sudden infant death syndrome *Curr Probl Pediatr 3* 3 1973

3 Beckwith J B *In* A B Bergman J B Beckwith & C D Ray (eds) *Sudden infant death syndrome* University of Washington Press Seattle and London 1970 p 17

4 Bergman A B Ray C G Pomeroy M A Wahl P W & Beckwith J B Studies of the sudden in fant death syndrome in King County Washington III Epidemiology *Pediatrics 49* 860 197?

5 Biering Sorensen F Jorgensen T & Hilden J Sudden infant death in Copenhagen 1956–71 I Infant feeding *Acta Paediatr Scand 67* 1?9 1978

6 Biering Sorensen F Jorgensen T & Hilden J Sudden infant death in Copenhagen 1956–71 II Social factors and morbidity *Acta Paediatr Scand* 68 1 19?9

7 Fedrick J Sudden unexpected death in infants in the Oxford Record Linkage Area An analysis with respect to time and place *Br J Prev Soc Med 27* 217 1973

8 Fedrick J Sudden unexpected death in infants in the Oxford Record Linkage Area The mother *Br J Prev Soc Med 28* 93 1974

9 Fedrick J Sudden unexpected death in infants in the Oxford Record Linkage Area Details of pregnancy delivery and abnormality in the infant *Br J Prev Soc Med 28* 164 1974

10 Froggatt P Epidemiologic aspects of the Northern Ireland study *In* Bergman A B Beckwith J B & Ray C D (eds) *Sudden infant death syndrome* University of Washington Press Seattle and London 1970 p 3?

11 Froggatt P Lynas M A & MacKenzie G Epi demiology of sudden unexpected death in infants (cot death) in Nothern Ireland *Br J Prev Soc Med 25* 119 1971

1? Froggatt P Lynas M A & Marshall T K Sud den unexpected death in infants (cot death) Report of a collaborative study in Northern Ireland *Ulster Med J 40* 116 1971

13 Jørgensen T Biering Sørensen F & Hilden J Sudden infant death in Copenhagen 1956–71 IV In fant development To be published

14 Kraus A S Steele R Thompson M G & deGros-bois P Further epidemiologic observations on sud den unexpected death in infancy in Ontario *Can J Publ Health 62* 210 1971

15 Kraus J F & Borhani N O Post neonatal sudden unexplained death in California A cohort study *Am J Epidemiol 95* 497 1972

16 Ministry of Health Enquiry into sudden death in in fancy *Reports on Public Health and Medical Sub-jects No 113* Her Majesty s Stationery Office Lon don 1965

17 Naeye R L Ladis B & Drage J S Sudden infant death syndrome A prospective study *Am J Dis Child 130* 1?07 1976

18 Protestos C D Carpenter R G McWeeny P M & Emery J L Obstetric and perinatal histories of children who died unexpectedly (cot death) *Arch Dis Child 48* 835 1973

19 Raring R H SIDS—A note on age distribution of the syndrome *In* R R Robinson (ed) *SIDS 1974 proceedings of the Francis E Camps international symposium on sudden and unexpected deaths in in fancy* The Canadian Foundation for the study of infant deaths Toronto 1974 p 151

?0 Richards I D G & McIntosh H T Confidential inquiry into ?66 consecutive infant deaths *Arch Dis Child 47* 697 1972

21 Spiers P S Previous fetal loss and risk of sudden infant death syndrome in subsequent offspring *Am J Epidemiol 103* 355 1976

?? Steele R & Langworth J T The relationship of antenatal and postnatal factors to sudden unexpected death in infancy *Canad Med Assoc J 94* 1165 1966

23 Steele R Kraus A S & Langworth J T Sudden unexpected death in infancy in Ontario Part I Meth odology and findings related to the host *Can J Publ Health 58* 359 1967

Age at death

In all materials the distribution by age at death (1, 2 7, 11, 15, 20 22 25) has shown the same tendency viz few deaths during the first month of life, a peak at two to four months followed by a rapid fall Accordingly by far the greater part of the deaths occur within the first six months ranging in the available series from 65% to 100%

Raring (19) pooled age at death data from seven materials This gave a reasonably smooth curve which he interpreted as being log normal In his opinion this supported the theory that SIDS was a separate distinct disease entity

If SIDS is to be considered a disease entity it would be reasonable to compare it with the age at death curves for other disease groups In our Fig 2 it is very similar to the curve for deaths from respiratory tract infections This indeed agrees with the findings that (*a*) respiratory symptoms are quite common during the days preceding death and that (*b*) there is an accumulation of deaths during the cold months (6) This is supported by an official report from England (16) Richards & McIntosh (20) compared the age at death for SIDS cases with those for children dying of gastroenteritis (n= 28) and pneumonia (n=36) without being able to find any striking resemblance The SIDS curve differs widely from that for other infant deaths considered together (Fig 2) as is indeed well known (15)

An author from New Zealand (24) like us has reported that premature SIDS cases die later than mature ones but the difference is not significant In two publications (4 10) it is merely mentioned without giving any figures that the age at death of prematures and matures does not differ significantly These reflections are of importance in assessing the theory that SIDS occurs during a vulnerable period of physiological development corresponding to the characteristic narrow age distribution at death (11 12) because if premature cases die later than mature cases this phenomenon may be interpreted to reflect that prematures take longer to reach the vulnerabl period

Circumstances at death and time when found dead

That 13% died while sharing a bed with a adult supports the idea of instantaneous deatl without any death struggle (4) It would be o interest to compare the position when foun with these and other infants normal sleepin position and the position of other dead infants This has been done but the results hav not been consistent (2 4 12)

Other materials (2 4 12 23) support ou finding that most of the infants are found dea in the morning or less often in the afternoon These hours correspond to the infants dyin in their sleep during the night or during th midday nap The sex difference in this respect like the other sex differences is not explic able

CONCLUSIONS

In this and in the preceding paper (6) we hav confirmed that SIDS families are under privi leged as regards several social factors and pe rinatal factors of social nature We have alsc pointed out that some of the factors can ex plain each other and we have emphasized the tendency for certain unfavourable factors not to accumulate any longer in SIDS cases and families We have quoted the theory that a physiologically vulnerable period (11 12) underlies SIDS which is suggested by the age curve and that various (unknown?) external factors need be only small to cause the in fant s death if they occur during the vulner able period In view of this theory one might think that protection against or improvement of external factors would—at least to some ex tent—reduce the incidence of SIDS One ex ternal factor might be for instance a trivial infection which again might be elicited by various influences of social nature—including older siblings as possible vectors

But how is the constant incidence of SIDS

Acta Pædiatr Scand 68 23–27 1979

IgG SUBCLASS LEVELS IN INFANCY AND CHILDHOOD

VIVI ANNE OXELIUS

From the Department of Paediatrics University Hospital Lund Sweden

ABSTRACT Oxelius V (Department of Paediatrics University Hospital Lund Sweden) IgG subclass levels in infancy and childhood Acta Paediatr Scand 68 23 1979 —The concentrations of IgG1 IgG2 IgG3 and IgG4 were determined by electroimmunoassay in 10 pairs of maternal and cord sera and in sera of 162 healthy children aged 6 weeks to 15 years Specific rabbit antisera against the IgG subclasses were used The content of the normal serum pool WHO 67/97 was used as reference The mean value standard deviation and normal range of each IgG subclass were calculated for each age group and compared with the adult values All IgG subclasses were present in cord serum except for IgG4 in those cases where also the maternal serum lacked demonstrable IgG4 The IgG subclasses followed the pattern of total IgG with a fall during the first 3–6 months and a subsequent gradual rise with age The IgG1 and IgG3 levels rose faster with age than IgG2 and IgG4 Adult levels were not reached before puberty No IgG4 was detectable in 12–21% of the children above 7 years of age

KEY WORDS Serum IgG subclasses childhood

Quantification of immunoglobulins in serum is one of the parameters used to assess the immunological competence of an individual The normal range and variation of concentrations of the different immunoglobulin classes in serum is wide in children and in adults It is further known that the concentrations are lower in childhood than in adult age and that the age at which the adult level is reached varies from class to class (1 2 3 4 19 20 23)

Human IgG consists of four subclasses based on antigenic differences in their heavy polypeptide chains (22) The proportions of the IgG subclasses in a normal serum pool are IgG1 IgG2 IgG3 IgG4 60 9% 29 6% 5 3% 4 2% (11 18) Imbalance of IgG subclasses and deficiency of IgG subclasses have been reported in children with recurrent infections (14 17 21 25) Only a few studies are available on the levels of the IgG subclasses in healthy children (5 6 9 10) The purpose of this investigation was to assess the variation of IgG subclass levels in normal Swedish children from birth to 15 years of age

MATERIALS AND METHOD

Umbilical cord blood from 10 fullterm newborns and venous blood from their mothers were examined Another 10 cord samples from fullterm newborns and venous blood samples from 162 children aged 6 weeks to 15 years were also examined The main part of the older children were inpatients admitted to the Children s Department of Surgery because of hernia inguinalis hydrocele testis hernia femoralis and other surgical non immunological diseases One criterion for admission was that the children should be free from infection Only patients with an E S R below 10 mm/hr were included in the present material Venous blood samples were also obtained from healthy infants from the Baby Wellfare Center Age distribution within the groups is shown in Table 1 Venous blood from 20 normal adults was included in the material The serum samples obtained were kept at −20°C until analysed

A normal serum pool of 400 blood donors with an IgG content of 178 I U /ml was used The reference was the WHO pool 67/97 with an IgG content of 96 I U /ml In the reference serum pool 67/97 the IgG subclasses have been determined to 5 1 g/l for IgG1 2 5 g/l for IgG2 0 55 g/l for IgG3 and 0 35 g/l for IgG4 (18) The values in g/l given in this article were according to the values reported for the reference WHO pool 67/97 The IgG subclasses of our normal serum pool (100%) were calculated to IgG1 6 8 g/l IgG2 3 33 g/l IgG3 0 73 g/l and IgG4 0 47 g/l

Specific IgG subclass antisera prepared in rabbits as described earlier (15) were used The specificity of antisera were tested against purified myeloma proteins of all

24 Tonkin S Epidemiology of SIDS in Auckland New Zealand *In* R R Robinson (ed) *SIDS 1974 proceedings of the Francis E Camps international symposium on sudden and unexpected deaths in infancy* The Canadian Foundation for the study of infant deaths Toronto 1974 p 169
25 Valdes Dapena M A Sudden and unexpected death in infancy A review of the world literature 1954–66 *Pediatrics 39* 123 1967
26 Valdes Dapena M McGovern J A Birle L J & Auerbach V H Changes in annual incidence rates of sudden unexpected deaths among infants in Philadelphia 1960–72 *J Pediatr 84* 776 1974

Submitted March 11 1978
Accepted May 19 1978

(J H) Institute of Human Genetics
University of Copenhagen
Rådmandsgade 71 A
DK 2200 Copenhagen N
Denmark

Acta Pædiatr Scand 68 23–27 1979

IgG SUBCLASS LEVELS IN INFANCY AND CHILDHOOD

VIVI ANNE OXELIUS

From the Department of Paediatrics University Hospital Lund Sweden

ABSTRACT Oxelius V (Department of Paediatrics University Hospital Lund Sweden) IgG subclass levels in infancy and childhood Acta Paediatr Scand 68 23 1979.—The concentrations of IgG1 IgG2 IgG3 and IgG4 were determined by electroimmunoassay in 10 pairs of maternal and cord sera and in sera of 162 healthy children aged 6 weeks to 15 years Specific rabbit antisera against the IgG subclasses were used The content of the normal serum pool WHO 67/97 was used as reference The mean value standard deviation and normal range of each IgG subclass were calculated for each age group and compared with the adult values All IgG subclasses were present in cord serum except for IgG4 in those cases where also the maternal serum lacked demonstrable IgG4 The IgG subclasses followed the pattern of total IgG with a fall during the first 3–6 months and a subsequent gradual rise with age The IgG1 and IgG3 levels rose faster with age than IgG2 and IgG4 Adult levels were not reached before puberty No IgG4 was detectable in 12–21 % of the children above 7 years of age

KEY WORDS Serum IgG subclasses childhood

Quantification of immunoglobulins in serum is one of the parameters used to assess the immunological competence of an individual The normal range and variation of concentrations of the different immunoglobulin classes in serum is wide in children and in adults It is further known that the concentrations are lower in childhood than in adult age and that the age at which the adult level is reached varies from class to class (1 2 3 4 19 20 23)

Human IgG consists of four subclasses based on antigenic differences in their heavy polypeptide chains (22) The proportions of the IgG subclasses in a normal serum pool are IgG1 IgG2 IgG3 IgG4 60 9% 29 6% 5 3% 4 2% (11 18) Imbalance of IgG subclasses and deficiency of IgG subclasses have been reported in children with recurrent infections (14 17 21 25) Only a few studies are available on the levels of the IgG subclasses in healthy children (5 6 9 10) The purpose of this investigation was to assess the variation of IgG subclass levels in normal Swedish children from birth to 15 years of age

MATERIALS AND METHOD

Umbilical cord blood from 10 fullterm newborns and venous blood from their mothers were examined Another 10 cord samples from fullterm newborns and venous blood samples from 162 children aged 6 weeks to 15 years were also examined The main part of the older children were inpatients admitted to the Children s Department of Surgery because of hernia inguinalis hydrocele testis hernia femoralis and other surgical non immunological diseases One criterion for admission was that the children should be free from infection Only patients with an E S R below 10 mm/hr were included in the present material Venous blood samples were also obtained from healthy infants from the Baby Wellfare Center Age distribution within the groups is shown in Table I Venous blood from 20 normal adults was included in the material The serum samples obtained were kept at −20°C until analysed

A normal serum pool of 400 blood donors with an IgG content of 128 I U /ml was used The reference was the WHO pool 67/97 with an IgG content of 96 I U /ml In the reference serum pool 67/97 the IgG subclasses have been determined to 5 1 g/l for IgG1 2 5 g/l for IgG2 0 55 g/l for IgG3 and 0 35 g/l for IgG4 (18) The values in g/l given in this article were according to the values reported for the reference WHO pool 67/97 The IgG subclasses of our normal serum pool (100%) were calculated to IgG1 6 8 g/l IgG2 3 33 g/l IgG3 0 73 g/l and IgG4 0 47 g/l

Specific IgG subclass antisera prepared in rabbits as described earlier (15) were used The specificity of antisera were tested against purified myeloma proteins of all

24 Tonkin S Epidemiology of SIDS in Auckland New Zealand *In* R R Robinson (ed) *SIDS 1974 proceedings of the Francis E Camps international symposium on sudden and unexpected deaths in infancy* The Canadian Foundation for the study of infant deaths Toronto 1974 p 169
25 Valdes Dapena M A Sudden and unexpected death in infancy A review of the world literature 1954–66 *Pediatrics* 39 123 1967
26 Valdes Dapena M McGovern J A Birle L J & Auerbach V H Changes in annual incidence rates of sudden unexpected deaths among infants in Philadelphia 1960–72 *J Pediatr* 84 776 1974

Submitted March 11 1978
Accepted May 19 1978

(J H) Institute of Human Genetics
University of Copenhagen
Rådmandsgade 71 A
DK 2200 Copenhagen N
Denmark

34		
an	S D	Range
9	0 18	<0 01–0 47
4	0 14	<0 01–0 33
6	0 04	<0 01–0 14
0	0	<0 01
3	0 19	<0 01–0 65
25	0 3?	<0 01–1 16
3?	0 36	<0 01–1 1
3	0 25	<0 01–0 84
41	0 33	<0 01–1 21
71	0 54	<0 01–1 68
3?	0 ?7	<0 01–0 83
55	0 63	<0 01–? 91

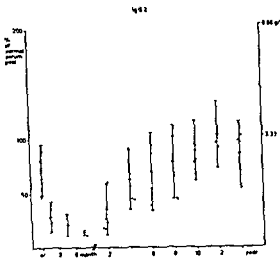


Fig 3 The concentration relative to that of a normal serum pool (IgG 1,8 I U) and in g/l of IgG2 in each individual compared with mean value ±1 S D in age groups

strable IgG4 The mean value for IgG4 as well as for the other IgG subclasses then slowly increased with age The concentration of IgG1 and IgG3 reached 50% of average adult con centration before 3 years of age those of IgG2 and IgG4 after this age (non detectable IgG4 was regarded as 0 005 g/l) The concentration of IgG4 varied most and that of IgG1 least Normal adult values were not reached by the oldest age group tested at 13–15 years of age IgG4 was still non detectable in 12–21% of the children above 7 years of age

DISCUSSION

The electroimmunoassay has proved a sen sitive and accurate method for evaluation of the content of IgG subclasses in human serum (15) Good agreement was found between our results and those on record (6 9) The normal ranges of variation of IgG subclasses at dif ferent ages were estimated in order to facilitate the diagnosis of suspected IgG subclass de ficiency conditions in children

All the four IgG subclasses were found in cord sera However when the mother has no serum IgG4 the newborn constantly had no IgG4 in cord serum This was evidence of the passive transfer of IgG4 across the placenta

The IgG subclasses were not found in the same proportions in cord serum as in the ma ternal serum This means that the difference in concentrations between cord serum and maternal serum could not be explained en tirely by plasma volume differences at birth In every case the IgG1 cord serum level was higher than the level in the maternal serum This is consistent with an active placental transport of IgG1 as is already described for total IgG (8) The IgG2 IgG3 and IgG4 cord serum levels were either higher or lower than the corresponding maternal serum levels These findings indicate differences in trans port mechanisms between the IgG subclasses A selective transfer of different Gm factors across the placenta was shown already by Hunger & Thierbach (7) The child s own IgG synthesis starts during intra uterine life (13) but judging from studies of Gm factors the amount produced is negligible compared with the amount originating from the mother Con cerning the metabolism of the four IgG sub classes it is roughly equal except for IgG3 (12) which has a biological half life of only one third of that of any of the other IgG sub classes

All the serum IgG subclasses increase slow

Table 1 *IgG subclass levels in g/l in 182 healthy children compared with those in adults*

		IgG1			IgG2			IgG3		
Age	No	Mean	S D	Range	Mean	S D	Range	Mean	S D	Range
Cord sera	20	6 02	1 23	4 35–10 84	2 38	0 76	1 43–4 53	0 59	0 32	0 27–1 46
0–2 months	7	3 67	1 02	2 18–4 96	0 93	0 47	0 40–1 67	0 16	0 08	0 04–0 73
3–5 months	9	3 06	0 82	1 43–3 94	0 70	0 33	0 23–1 47	0 30	0 29	0 04–1 00
6–8 months	8	2 99	0 75	1 90–3 88	0 47	0 07	0 37–0 60	0 32	0 16	0 17–0 67
9 months–2 years	24	4 49	1 09	2 86–6 80	1 17	0 77	0 30–3 27	0 42	0 31	0 13–0 87
3–4 years	22	5 58	1 02	3 81–8 84	2 03	0 90	0 70–4 43	0 51	0 22	0 17–0 90
5–6 years	20	4 83	1 36	2 92–8 16	2 27	1 17	0 83–5 13	0 49	0 32	0 08–1 11
7–8 years	17	5 71	1 16	4 22–8 02	2 57	1 10	1 13–4 80	0 61	0 34	0 15–1 33
9–10 years	20	6 73	1 22	4 56–9 38	2 90	0 90	1 63–5 13	0 67	0 21	0 26–1 13
11–12 years	16	6 46	1 29	4 56–9 52	3 37	1 00	1 47–4 93	0 64	0 44	0 17–1 79
13–14 years	19	6 39	1 56	3 47–9 93	2 80	0 97	1 40–4 40	0 69	0 34	0 23–1 17
Adults	20	7 55	2 45	4 22–12 92	3 80	1 50	1 17–7 47	0 73	0 26	0 41–1 79

Amounts less than 0 01 g/l regarded as 0 005 g/l

IgG subclasses in Ouchterlony technique and immunoelectrophoresis (15)

For quantitation of IgG subclasses in sera the electro immunoassay was used as described earlier (15) The sensitivity of the method is about 0 01 g/l for all IgG subclasses

RESULTS

All IgG subclasses were present in the cord sera except for IgG4 in those cases where the mother had no demonstrable IgG4 (<0 01 g/l) in the serum The IgG1 serum levels were regularly higher in the newborns than in the mothers The serum IgG2 IgG3 and IgG4 concentrations in the newborns were either higher or lower than those in the mothers (Fig 1)

The IgG subclass values found for different age groups are given in Figs 2–5 and in Table 1 The lowest values were noted in the age groups 0–2 to 6–8 months In the latter age group none of the children had any demon

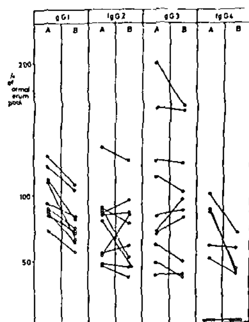


Fig 1 The concentration of IgG subclasses in 10 cord (A) and maternal (B) sera

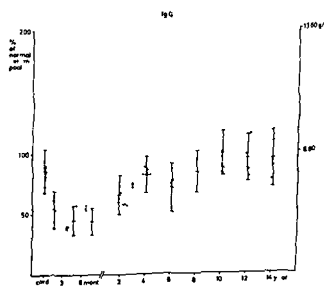


Fig 2 The concentration relative to that of a normal serum pool (IgG 128 I U) and in g/l of IgG1 in each individual compared with mean value ±1 S D in age groups

Lovisa s Foundation Alfred Österlund s Foundation and the Medical Faculty University of Lund

REFERENCES

1 Allansmith M McClellan R H Butterworth M & Maloney I R The development of immunoglobulin levels in man *J Ped atr 72* 276 1968
? Berg T & Johansson S G O Immunoglobulin levels during childhood with special regard to IgE *Acta Paediatr Scand 58* 513 1969
3 Collins-Williams C Tkachyk S J Toft B & Moscarello M Quantitative immunoglobulin levels (IgG IgA and IgM) in children *Int Arch Allergy 31* 94 1967
4 Geny B Rodary C Griscelli P & Mozziconacci P Immunoglobulin D (IgD) in childhood I Maturation of serum IgD study of a population of healthy children *Biomedicine 20* 40 1974
5 Giessen M van der Freyee W Rossouw E & Loghem E van Qualitative and quantitative studies of IgG? globulins in individual human sera with an antiserum capable of differentiating between Gm(n+) and Gm(n−) proteins *Clin Exp Immunol 14* 127 1973
6 Giessen M van der Rossouw E Algra van Veen T Loghem E van Zegers B J M & Sander P C Quantification of IgG subclasses in sera of normal adults and healthy children between 4 and 1? years of age *Clin Exp Immunol 21* 501 1975
7 Hunger H & Thierbach V Gm(a) Bestimmungen bei Muttern und Neugeborenen *Blut 9* 385 1963
8 Kohler P F & Farr R S Elevation of cord over maternal IgG immunoglobulin Evidence for an active placental transport *Nature 210* 1070 1966
9 Morell A Skvaril F Hitzig W H & Barandun S IgG subclasses Development of the serum concentrations in normal infants and children *J Pediatr 80* 960 197?
10 Morell A Skvaril F Steinberg A G Loghem E van & Terry W D Correlations between the concentrations of the four subclasses of IgG and Gm allotypes in normal sera *J Immunol 108* 195 197?
11 Morell A Skvaril F & Barandun S *IgG subclasses der menschlichen Immunoglobuline* S Karger Basel Munchen Paris London New York and Sydney 1975
12 Morell A Terry W D & Waldmann T A Metabolic properties of IgG subclasses in man *J Clin Invest 49* 673 1970
13 Mårtensson L & Fudenberg H H Gm genes and γG-globulin synthesis in the human fetus *J Immunol 94* 514 1965
14 Oxelius V A Chronic infections in a family with hereditary deficiency of IgG? and IgG4 *Clin Exp Immunol 17* 19 1974
15 Oxelius V A Crossed immunoelectrophoresis and electroimmuno assay of human IgG subclasses *Acta Path Microbiol Scand Sect C 86* 109 1978
16 Peltola H Kayhty H Sivonen A & Makelä P H *Haemophilus influen ae* type b capsular polysaccharide vaccin in children A double blind field study of 100000 vaccinees 3 months to 5 years of age in Finland *Pediatrics 60* 730 1977
17 Schur P H Borel H Gelfand E W Alper C & Rosen F S Selective gamma-G globulin deficiencies in patients with recurrent pyogenic infections *N Engl J Med 283* 631 1970
18 Steinberg A G Morell A Skvaril F & Loghem E van The effect of Gm(?3) on the concentration of IgG? and IgG4 in normal human serum *J Immunol 110* 164 1973
19 Stiehm E R & Fudenberg H H Serum levels of immunoglobulins in health and disease *Pediatrics 37* 715 1966
?0 Stoop J W Zegers B J M Sander P C & Ballieux R E Serum immunoglobulin levels in healthy children and adults *Clin Exp Immunol 4* 101 1969
?1 Terry W D Variations in the subclasses of IgG *In* R A Good & D Bergsma (eds) *Immunologic deficiency diseases in man Birth defects* The National Foundation 1968 p 357
? Terry W D & Fahey J L Subclasses of human gamma$_2$-globulin based on differences on the heavy polypeptide chains *Science 20* 870 1964
?3 Uffelman J A Engelhard W E & Jolliff C R Quantitation of immunoglobulins in normal children *Clin Chim Acta 28* 185 1970
?4 Yount W J Dorner M M Kunkel H G & Kabat E A Studies on human antibodies VI Selective variations in subgroup composition and genetic markers *J Exp Med 127* 633 1968
?5 Yount W J Hong R Seligman M Good R A & Kunkel H G Imbalances of gammaglobulin subgroups and gene defects in patients with primary hypogammaglobulinemia *J Clin Invest 49* 1957 1970

Submitted April 1 1978
Accepted May ?5 1978

Department of Paediatrics
University Hospital
2?185 Lund
Sweden

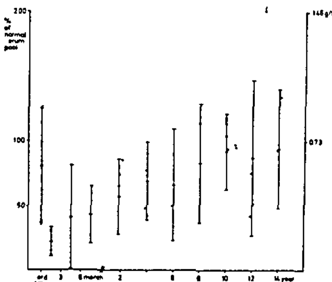


Fig 4 The concentration relative to that of a normal serum pool (IgG 128 I U) and in g/l of IgG3 in each individual compared with mean value ±1 S D in age groups

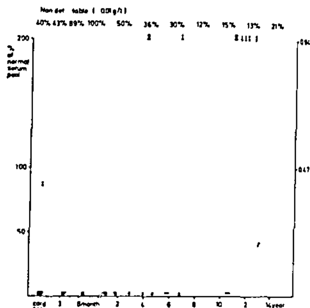


Fig 5 The concentration relative to that of a normal serum pool (IgG 128 I U) and in g/l of IgG4 in each individual compared with mean value ±1 S D in age groups The frequency of individuals lacking IgG4 (IgG4 0 01 g/l) is given for each age group Amounts less than 0 01 g/l regarded as 0 005 g/l

ly during childhood Like others (9) we found that IgG1 and IgG3 increased faster with age than did IgG2 and IgG4 This means that the cell population responsible for each IgG subclass mature at different ages of the growing child It is well known that specific antibodies belong to different IgG subclasses (24) The young child has not full immunological competence to make antibodies when stimulated for example by *Haemophilus influenzae* type b capsular polysaccharide vaccine (16) One of the reasons could be the delay in the synthesis of IgG2 and IgG4 We have earlier shown that antibodies to *Haemophilus influenzae* type b polysaccharide could not be found in patients with IgG2 and IgG4 deficiency (14)

The demonstrable IgG4 varied widely within each age group from zero to very high values Even in the higher age groups IgG4 was still undetectable in 12–21% IgG4 and IgD are quantitatively comparable Both IgG4 and IgD are found in relatively low concentration in serum both are undetectable in about 25% of normal individuals and both have a wide normal range of variation

IgG4 deficiency was shown to be very common in normal individuals IgG1 is quantitatively the biggest of the IgG subclasses IgG1 deficiency should therefore be suspected when a low IgG value is found IgG2 or IgG3 deficiency could be found in patients with low or normal serum IgG However it should be born in mind that IgG subclass deficiencies could be found also in patients with high IgG values IgG subclass deficiencies should also be suspected when there is a restricted heterogeneity of immunoglobulins by electrophoresis of serum or when a deficiency of specific antibodies in serum can be proved Because of continuously increasing serum levels in childhood it is advisable to repeat IgG subclass determinations after an interval of some months

ACKNOWLEDGEMENTS

I thank Mrs Ann Margreth Carlsson for skilful technical assistance This work was supported by Crown Princess

Lovisa s Foundation Alfred Österlund's Foundation and the Medical Faculty University of Lund

REFERENCES

1 Allansmith M McClellan R H Butterworth M & Maloney J R The development of immunoglobulin levels in man *J Pediatr 72* 276 1968
2 Berg T & Johansson S G O Immunoglobulin levels during childhood with special regard to IgE *Acta Paediatr Scand 58* 513 1969
3 Collins Williams C Tkachyk S J Toft B & Moscarello M Quantitative immunoglobulin levels (IgG IgA and IgM) in children *Int Arch Allergy 31* 94 1967
4 Geny B Rodary C Griscelli P & Mozzi onacci P Immunoglobulin D (IgD) in childhood I Maturation of serum IgD study of a population of healthy children *Biomedicine 20* 40 1974
5 Giessen M van der Freyee W Rossouw E & Loghem E van Qualitative and quantitative studies of IgG2 globulins in individual human sera with an antiserum capable of differentiating between Gm(n+) and Gm(n−) proteins *Clin Exp Immunol 14* 127 1973
6 Giessen M van der Rossouw E Algra van Veen T Loghem E van Zegers B J M & Sander P C Quantification of IgG subclasses in sera of normal adults and healthy children between 4 and 12 years of age *Clin Exp Immunol 21* 501 1975
7 Hunger H & Thierbach V Gm(a) Bestimmungen bei Muttern und Neugeborenen *Blut 9* 385 1963
8 Kohler P F & Farr R S Elevation of cord over maternal IgG immunoglobulin Evidence for an active placental transport *Nature 210* 1070 1966
9 Morell A Skvaril F Hitzig W H & Barandun S IgG subclasses Development of the serum concentrations in normal infants and children *J Pediatr 80* 960 197
10 Morell A Skvaril F Steinberg A G Loghem E van & Terry W D Correlations between the concentrations of the four subclasses of IgG and Gm allotypes in normal sera *J Immunol 108* 195 1972
11 Morell A Skvaril F & Barandun S *IgG subclasses der menschlichen Immunoglobuline* S Karger Basel Munchen Paris London New York and Sydney 1975
12 Morell A Terry W D & Waldmann T A Metabolic properties of IgG subclasses in man *J Clin Invest 49* 673 1970
13 Mårtensson L & Fudenberg H H Gm genes and γG-globulin synthesis in the human fetus *J Immunol 94* 514 1965
14 Oxelius V A Chronic infections in a family with hereditary deficiency of IgG2 and IgG4 *Clin Exp Immunol 17* 19 1974
15 Oxelius V A Crossed immunoelectrophoresis and electroimmuno assay of human IgG subclasses *Acta Path Microbiol Scand Sect C 86* 109 1978
16 Peltola H Kayhty H Sivonen A & Makela P H *Haemophilus influenzae* type b capsular polysaccharide vaccin in children A double blind field study of 100000 vaccinees 3 months to 5 years of age in Finland *Pediatrics 60* 730 1977
17 Schur P H Borel H Gelfand E W Alper C & Rosen F S Selective gamma-G globulin deficiencies in patients with recurrent pyogenic infections *N Engl J Med 283* 631 1970
18 Steinberg A G Morell A Skvaril F & Loghem E van The effect of Gm(23) on the concentration of IgG2 and IgG4 in normal human serum *J Immunol 110* 1642 1973
19 Stiehm E R & Fudenberg H H Serum levels of immunoglobulins in health and disease *Pediatrics 37* 715 1966
20 Stoop J W Zegers B J M Sander P C & Ballieux R E Serum immunoglobulin levels in healthy children and adults *Clin Exp Immunol 4* 101 1969
21 Terry W D Variations in the subclasses of IgG *In* R A Good & D Bergsma (eds) *Immunologic deficiency diseases in man Birth defects* The National Foundation 1968 p 357
22 Terry W D & Fahey J L Subclasses of human gamma$_2$-globulin based on differences on the heavy polypeptide chains *Science 20* 870 1964
23 Uffelman J A Engelhard W E & Jolliff C R Quantitation of immunoglobulins in normal children *Clin Chim Acta 28* 185 1970
24 Yount W J Dorner M M Kunkel H G & Kabat E A Studies on human antibodies VI Selective variations in subgroup composition and genetic markers *J Exp Med 127* 633 1968
25 Yount W J Hong R Seligman M Good R A & Kunkel H G Imbalances of gammaglobulin subgroups and gene defects in patients with primary hypogammaglobulinemia *J Clin Invest 49* 1957 1970

Submitted April 1 1978
Accepted May 25 1978

Department of Paediatrics
University Hospital
2185 Lund
Sweden

Acta Pædiatr Scand 68 29–31 1979

BREAST MILK IRON—A DECLINING CONCENTRATION DURING THE COURSE OF LACTATION

MARTTI A SIIMES ERKKI VUORI and PEKKA KUITUNEN

From the Children s Hospital and the Department of Public Health Science University of Helsinki Helsinki Finland

ABSTRACT Siimes M A Vuori E and Kuitunen P (Children s Hospital and Department of Public Health Science University of Helsinki Helsinki Finland) Breast milk iron—a declining concentration during the course of lactation Acta Pædiatr Scand 68 29 1979 —The present investigation is the first longitudinal study of the concentration of iron in breast milk and is based on 229 milk samples obtained from 27 mothers during their period of lactation up to 9 months The samples were collected at the beginning and at the end of each feed during a 24-h period to reflect as accurately as possible the actual concentration of iron The median value declined during the course of lactation from 0 6 to 0 3 mg/l with a large range of values The results indicate that the concentration is lower than is generally stated or is unusually low in Finnish mothers As a consequence some infants may require iron supplementation during prolonged breast feeding although in general the high bioavailability of breast milk iron prevents the de elopment of iron deficiency

KEY WORDS Human milk lactation iron

The available data on the concentration of iron in breast milk indicate that the mean values ranged from 0 6 to 1 3 mg/l in the 50 s (2 4 5) and from 0 6 to 1 0 mg/l in the 60 s (3 6) and from 0 2 to 0 8 mg/l in the 70 s (1 9–11) The declining concentration may reflect a real phenomenon or it may be due to differences in methods of assay and sampling At present the methods of determining iron by sensitive flame atomic absorption photometry (AAS) is generally considered valid However the methods of obtaining the milk samples may influence the results since various iron concentrations for milk samples are reported in the literature and this difference in concentration is greater than that of some other minerals (11) since diurnal variation (11) and declining concentrations due to the stage of lactation play a role (6)

We feel there is a need for a longitudinal study in which milk samples are obtained in order to reflect as closely as possible the actual intake of iron and that such samples should be adequately analyzed This question has attained actuality since the bioavailability of iron in breast milk has been observed to be unusually high i e of the magnitude of 50% in infancy (14) The increasing knowledge of the range of the concentrations and the availability of iron in breast milk may serve as a basis for deciding whether iron supplementation is necessary in prolonged breast fed infants

SUBJECTS AND METHODS

In the present study a group of 27 breast feeding mothers was followed up after a ? week period of lactation Milk aliquots of 8 ml were obtained at the beginning and at the end of each feed during a ?4 h period (17) This method of collection allowed the mothers to continue the breast feeding of their infants and eliminated the diurnal variation and partly the potential variation during a single feeding A total of 2?9 24 h samples from about 50–110 ml each were obtained during the nine months of lactation from 27 mothers Initially the samples were obtained at intervals of 1–2 weeks and after lactation of ? months every 3rd–4th week The number of breast feeding mothers gradually declined to 13 at 6 months of lactation By 9 months there were only two mothers left in the study Over 60% of the mothers received iron supplementation daily after their delivery for the period of the present study In ad

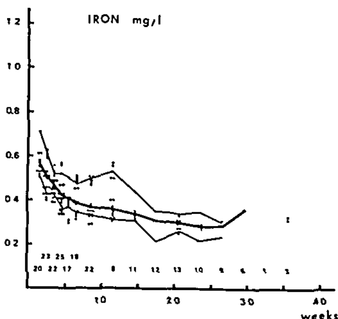


Fig. 1. Iron concentration in 229 breast milk samples obtained from 27 mothers during a 9 month period of lactation. The total range and the 25th, 50th and 75th percentiles are shown of samples at 2–7 weeks of lactation and three weekly thereafter. The mean values were 0.01–0.07 mg/l higher than the medians. Numerals indicate the number of samples in each interval of time.

dition, approximately half of the remaining mothers received iron medication irregularly.

The iron concentration in the milk samples frozen for a period of 2 to 6 weeks before analysis was determined from dry ash solution (17) by the flame AAS method (Perkin Elmer, model 300). Bovine liver, a reference material 1577 from the National Bureau of Standards, Washington D.C., was used as a control. Four determinations resulted in values ranging from 294 to 297 μg/g dry weight and was slightly above the certified value of 270±20 μg/g dry weight. A pooled milk sample was analyzed 11 times and resulted in a mean of 0.39 mg/l between series with a S.D. value of 0.01 mg/l.

RESULTS

The median concentration of iron was 0.56 mg/l in the breast milk samples at two weeks of lactation (Fig. 1). Thereafter the value gradually declined and reached a plateau at a value of approximately 0.3 mg/l after 5 months of lactation. The mean values were generally higher due to the skewed distribution of the values. There was a marked individual variation from the lowest single value of 0.11 mg/l to the highest one of 1.14 mg/l (Fig. 1). The variation in concentration was greater before 3 months of lactation and varied especially within the high values for this period as indicated by the relatively narrow 50% range in Fig. 1.

There was also a tendency to maintain the individual concentrations as indicated by the finding that 13 of the 23 mothers breast feeding in the third month the concentration of iron remained in the same initial quartile and additional 7 mothers remained within the range of ± one quartile of the initial value.

DISCUSSION

The present investigation is the first longitudinal study of the concentration of iron in breast milk. The results indicate that the concentration in one half of the cases is initially within the range of 0.52 and 0.69 mg/l and later during the course of prolonged lactation averages approximately 0.3 mg/l. The decrease in the concentration is in accordance with a similar pattern in the concentration of lactoferrin (7). On the other hand, mean values rather than medians have been used consistently in all previous studies (1–6, 9–11) which has resulted in overestimating the concentration of iron due to the skewed distribution of the values.

Stored iron (16) and the concentration of hemoglobin (13) in infants reach the low and the lowest level, respectively, by the age of 6 months, at the time when the concentration of iron in breast milk has also reached a low plateau as indicated in the present study. Following from the three aforementioned observations one may raise the question how long a period of time an infant can maintain an optimal iron nutrition while he is exclusively or mainly fed on breast milk. On the other hand, several recent reports indicate that the bioavailability of breast milk iron is unusually high (8, 13, 15) especially in exclusively breast fed infants (15). The available data also suggest that breast feeding up to a period of 6 months is not associated with a risk of iron de

ficiency (12) However such a risk might exist in some individual infants whose mother s milk iron concentration remains at a lower range of values in prolonged breast feeding or after an early introduction of solid foods

ACKNOWLEDGEMENT

Supported by a grant from the Yrjö Jahnsson Foundation Helsinki Finland

REFERENCES

1 Anon The composition of mature human milk *Report on Health and Social Subjects 12* Her Majesty s Stationery Office London 1977
2 Belavady B & Gopalan C Chemical composition of human milk in poor Indian women *Indian Jour Med Res 47* 234 1959
3 Cavell P A & Widdowson E M Intakes and excretions of iron copper and zinc in the neonatal period *Arch Dis Child 39* 496 1964
4 Feuillen Y M & Plumier M Iron metabolism in infants 1 The intake of iron in breast feeding and artificial feeding (milk and milk foods) *Acta Paediatr Scand 41* 138 1952
5 Kasanen A & Ekman E M The iron content of breast milk and its variations *Duodecim 71* 855 1955
6 Kleinbaum H Uber den Kupfergehalt der Nahrungsmittel des Kindes *Z Kinderheilkd 86* 655 196
7 Lonnerdal B Forsum E Gebre Medhin M & Hambraeus L Breast milk composition in Ethiopian and Swedish mothers II Lactose nitrogen and protein content *Am J Clin Nutr 29* 1134 1976
8 McMillan J A Landaw S A & Oski F A Iron sufficiency in breast fed infants and the availability of iron from human milk *Pediatrics 58* 686 1976
9 Murthy G K & Rhea U S Cadmium copper iron lead manganese and zinc in evaporated milk infant products and human milk *J Dairy Sci 54* 1001 1971
10 Nassi L Poggini G Vecchi C & Galvan P Notes on the zinc copper and iron content of human colostrum and milk *Minerva Pediatr 26* 832 1974
11 Picciano M F & Guthrie H A Copper iron and zinc contents of mature human milk *Am J Clin Nutr 29* 242 1976
12 Saarinen U M Need for iron supplementation in infants on prolonged breast feeding *J Pediatr 93* 177 1978
13 Saarinen U M & Siimes M A Developmental changes in red blood cell counts and indices of infant after exclusion of iron deficiency both by laboratory criteria and by continuous iron supplementation *J Pediatr 92* 412 1978
14 Saarinen U M Siimes M A & Dallman P R Iron absorption in infants High bioavailability of iron is indicated by the extrinsic tag method of iron absorption and by the concentration of serum ferritin *J Pediatr 91* 36 1977
15 Saarinen U M & Siimes M A Iron absorption from breast milk cow s milk and iron supplemented formula An opportunistic use of changes in total body iron determined by hemoglobin ferritin and body weight in 132 infants *Pediatr Res* (in press)
16 Siimes M A Addiego J E & Dallman P R Ferritin in serum Diagnosis of iron deficiency and iron overload in infants and children *Blood 43* 581 1974
17 Vuori E & Kuitunen P The concentrations of copper and zinc in human milk—a longitudinal study *Acta Paediatr Scand 68* 33 1979

Submitted March 4 1978
Accepted May 30 1978

(E V) Department of Public Health Science
University of Helsinki
Haartmaninkatu 3
SF-00 90 Helsinki 9
Finland

Acta Pædiatr Scand 68 33–37 1979

THE CONCENTRATIONS OF COPPER AND ZINC IN HUMAN MILK

A Longitudinal Study

ERKKI VUORI and PEKKA KUITUNEN

From the Department of Public Health Science and the Children s Hospital University of Helsinki Helsinki Finland

ABSTRACT Vuori E and Kuitunen P (Department of Public Health Science and Children s Hospital University of Helsinki Helsinki Finland) The concentrations of copper and zinc in human milk—a longitudinal study Acta Pædiatr Scand 68 33 1979,— Twenty-seven healthy Finnish mothers were followed during the course of their entire lactation period A total of 229 individual milk samples collected in the beginning and at the end of each feed during a 24-h period were obtained from the 2nd week to the 9th month of lactation The copper and zinc concentrations were determined by atomic absorption spectrophotometry The concentrations of the trace elements investigated were dependent on the stage of lactation The median copper and zinc concentrations decreased during the course of lactation from about 0 60 mg/l and 4 0 mg/l to 0 25 mg/l and 0 5 mg/l respectively The importance of considering the stage of lactation in the evaluation of the trace-element nutrition value of breast milk should be emphasized The calculated means of the concentrations of these trace-elements in mature human milk presented in the literature seem to overestimate the actual levels in prolonged lactation

KEY WORDS Human milk lactation copper zinc

Earlier studies on the trace element content of human milk have shown considerable variations both from mother to mother and in the case of copper and zinc also as regards the stage of lactation (17) According to available data the copper levels have ranged from 0 15 to 1 34 mg/l (1–3 5 7 11–15) and the zinc content from 0 65 to 5 3 mg/l (1 3–5 10 13–15) on the average

It has not been clarified to what extent these variable data really reflect individual variation and to what extent the variation may be due to the different stages of lactation or perhaps be due to the different methods of sampling The problem is best solved by means of a longitudinal study and therefore we have followed up 27 mothers during their entire lactation period and have determined the concentrations of copper and zinc in human milk

According to the current recommendations for infant feeding breast milk is preferred and during the first four to six months of life it should fulfill the nutritional needs of the infant (8) The trace element content of breast milk when adequately sampled and analyzed ought to serve as a guide in the assessment of the nutritional needs of trace elements for the infant

MATERIAL

Mothers

Forty three primipara were asked if they would be interested in taking part in the study Thirty seven interested mothers were recontacted after discharge A group of thirty mothers was obtained during April and May 1976 Two of the mothers were unable to supply milk samples and one other mother used a breast pump for collection and thus three mothers were excluded from the follow up All mothers were healthy well nourished and lived in the greater Helsinki area The mean age of the mothers was 28 years with a range of 20 to 35 years

Milk samples

A special bottle was designed for sampling the milk a widemouthed 125 ml polyethylene bottle having a scale on the side each division representing a volume of 8

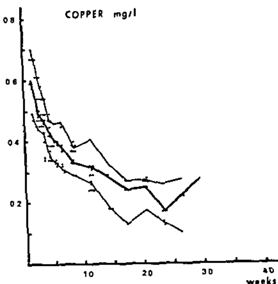


Fig 1 The average copper concentration of human milk during lactation The figure gives the total range median values and the 25th and the 75th percentiles after the ?nd week weekly and after the 7th week three weekly Total number of samples is ? 9

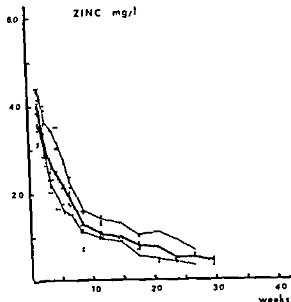


Fig 2 The average zinc concentration of human milk during lactation The figure gives the total range median values and the ?5th and the 75th percentiles after the 2nd week weekly and after the 7th week three weekly Total number of samples is ? 9

0 10–0 35 mg/l lower than the means of copper and zinc concentrations respectively

In the beginning of the study the median concentrations of copper and zinc were 0 60 mg/l and 4 0 mg/l The trace-element con centrations investigated showed to be depend ent on the stage of lactation (Figs 1–2) After 20 weeks of lactation the medians of copper and zinc values were about 0 25 mg/l for cop per and 0 5 mg/l for zinc The total ranges of the copper values were 0 03–0 75 mg/l and of zinc values 0 18–6 0 mg/l

Table 4 allows a comparison of the copper and zinc concentrations in human milk pre sented together with some earlier data

DISCUSSION

On the basis of this longitudinal study it can be stated that the earlier data on the average trace element content of human milk over estimate the actual levels in prolonged lacta tion mature human milk is said to contain 0 4 mg/l copper and 3–5 mg/l zinc on the aver age (6) However 50% of our mothers had a copper content in their milk under the given value after 7 weeks of lactation and all of them after 14 weeks and in the case of zinc after 4 and 9 weeks respectively This ob liquity is perhaps caused by the fact that most earlier studies have been made in the be ginning of lactation (Table 4) and perhaps the previous reports on the effect of the stage of lactation on the trace element content of human milk have not been sufficiently con vincing

When studying human milk the first and perhaps the most difficult stage is to get rep resentative samples In previous studies all authors have collected their samples avoid ing contamination but only a few give in formation on how the samples were actually taken e g before nursing the baby (15) Hytten has put forward very rigid require ments which a sample of milk has to fulfill a pooled sample representing the entire milk production of a period covering 24 hours (9) However in studies conducted outside the

Table 1 *The median copper and zinc concentrations of human milk and the number of samples from the 2nd to the 37th week of lactation*

Stage of lactation week	No of samples	Cu mg/l	Zn mg/l
2	20	0 60	4 0
3	23	0 49	3 0
4	22	0 47	2 5
5	25	0 42	2 4
6	17	0 40	2 1
7	18	0 38	1 9
8–10	22	0 33	1 3
11–13	18	0 32	1 1
14–16	11	0 28	0 95
17–19	12	0 24	0 78
20–22	13	0 25	0 75
23–25	10	0 17	0 49
26–28	9	0 22	0 52
29–31	6	0 27	0 44
32–34	1	0 24	0 42
35–37	2	0 25	0 48

ml The mothers were advised to wash their breasts with plain water and to dry them afterwards Before and after each feed the mothers took a sample (one interval on the scale) by manual milking directly into the sampling bottle This was repeated every time the mother fed her baby over a period of 24 hours Thus the final sample of milk obtained over a 24 h period consisted of foremilk and hindmilk in equal proportions This method of collecting human milk has been used earlier e g by Tarján et al (16)

During the first three months of lactation the mothers nursed their infants 5–7 times and later on 3–4 times during each 24 h period Instructions were given to keep the bottle refrigerated during the collection of the samples On the following day the bottle was taken to the laboratory for deep freezing At first the mothers supplied milk samples at intervals of 1–2 weeks and after two months at intervals of 3–4 weeks The mean number of samples supplied by the mothers was 8 6 with a range of 3–14 Thus the group of 27 mothers supplied 229 milk samples from the 2nd to the 37th week of lactation (Table 1) Samples from the last 6 weeks were excluded from the statistical analysis because the number of mothers and the number of milk samples supplied was small

This method of collecting milk samples was found to be convenient and it did not disturb lactation

METHODS

Milk samples which had been stored frozen from 2 to 6 weeks were warmed to body temperature in a water bath and mixed by gentle shaking Ten milliliters of milk were measured into covered 50 ml silica crucibles The samples were dried at 105 C overnight They were then carbonized in a muffle furnace at 250 C for 4–5 hours and subsequently ashed at 450 C for about 48 hours In the middle of the ashing procedure the crucibles were allowed to cool and 0 1 ml of conc HNO_3 (Suprapur® Merck) was added after which the temperature was again raised gradually to 450 C The ash was first dissolved with 0 5 ml of 6 M HCl (Suprapur® Merck) and then with 1 ml of 2 M HCl with gentle warming The crucibles and covers were rinsed several times with small volumes of deionized water and finally diluted to 5 ml with deionized water

The copper concentration in the milk sample was then determined by the flame Atomic Absorption Spectrophotometric (AAS) method (Perkin Elmer model 300) directly from the acid ash solution For the determination of zinc content a further dilution (1+9) with deionized water was needed

The accuracy of the method was tested by analyzing Bovine Liver Standard Reference Material No 1577 supplied by the National Bureau of Standards Washington D C (Table 2) and the precision of the method was established by repeated analysis of pooled human milk (Table 3)

Table 2 *Accuracy of the method*

NBS Bovine Liver Standard Reference Material 1577 4 determinations μg/g dry weight

	Mean	Range	Certified value
Cu	200	198–200	193±10
Zn	140	138–141	130±10

RESULTS

Instead of using means and standard deviations as previously given by earlier authors we have expressed our results by giving the total ranges the 25th and 75th percentiles and median values because the distribution of the trace element concentrations was found to be skewed However the differences between the median and mean values were not great the medians being 0 00–0 03 mg/l higher and

Table 3 *Precision of the method*

Between series calculated from 11 samples of pooled human milk mg/l

	Mean	S D
Cu	0 41	0 02
Zn	2 5	0 1

75th percentiles Picciano & Guthrie also found the skewed distributions of copper and zinc in their material but in both cases towards lower values (15) In the light of the present longitudinal study the stage of lactation must be emphasized They had taken samples from the 6th to 12th weeks of lactation and according to our results the median of the copper content diminished from about 0 4 to 0 3 mg/l during the given period (Table 1) Thus the opposite skewed distribution might be caused by the preponderance of mothers who had nursed for a longer period

The copper and zinc concentrations of human milk presented here are compatible with that of most previous reports when the stage of lactation and the number of samples are taken into account (Table 4) The present results suggest that no single value can be given for the concentrations of trace elements in human milk without relating it to the stage of lactation As regards zinc and copper no mature milk seems to exist before the 5th–6th month of lactation Earlier calculated means of the concentrations of trace elements of mature human milk as presented in the literature seem to overestimate the actual levels Thus these values do not accurately reflect the supply of copper and zinc derived from human milk during prolonged breast feeding but the actual levels of trace elements as related to each stage of lactation should serve as a basis for determining the quantity of trace elements supplied to the infant

ACKNOWLEDGEMENT

Supported by a grant from the Yrjo Jahnsson Foundation Helsinki Finland

REFERENCES

1 Anon The composition of mature human milk *Report on Health and Social Subjects 12* Her Majesty s Stationery Office London 1977

2 Beck A B see Underwood (17) p 74

3 Belavady B & Gopalan C Chemical composition of human milk in poor Indian women *Indian Jour Med Res 47* 234 1959

4 Berfenstam R Studies on blood zinc A clinical and experimental investigation into the zinc content of plasma and blood corpuscles with special reference to infancy *Acta Paediatr Scand 41* 105 1952

5 Cavell P A & Widdowson E M Intakes and excretions of iron copper and zinc in the neonatal period *Arch Dis Child 39* 496 1964

6 Fomon S J *Infant nutrition* W B Saunders Company Philadelphia 1974 2nd ed

7 Grebennikov E P Soroka V R & Sabadash E V Trace elements in human and animal milk *Fed Proc 23* 461 1964

8 Hambraeus L Proprietary milk versus human breast milk in infant feeding A critical appraisal from the nutritional point of view *Pediatr Clin North Am 24* 17 1977

9 Hytten F E Clinical and chemical studies in human lactation *Br Med J I* 175 1954

10 Jensen R I Thomas L N Bergman K E Filer L J Jr & Fomon S J 197? see Fomon (6) p 364–365

11 Kleinbaum H Uber den Kupfergehalt der Nahrungsmittel des Kindes *Z Kinderheilkd 86* 655 196?

12 Munch Petersen P On the copper content in mother's milk before and after intravenous copper administration *Acta Pediatr Scand 9* 378 1950

13 Murthy G K & Rhea U S Cadmium copper iron lead manganese and zinc in evaporated milk infant products and human milk *J Dairy Sci 54* 1001 1971

14 Nassi L Poggini G Vecchi C & Galvan P Notes on the zinc copper and iron content of human colostrum and milk *Minerva Pediatr 26* 832 1974

15 Picciano M F & Guthrie H A Copper iron and zinc contents of mature human milk *Am J Clin Nutr 29* 24? 1976

16 Tarján R Kramer M Szoke S & Lindner K The effect of different factors on the composition of human milk and its variation I *Nutr Diet 5* 1 1963

17 Underwood E J *Trace elements in human and animal nutrition* Academic Press New York 1971 3rd ed

Submitted March 4 1978
Accepted May 30 1978

(E V) Department of Public Health Science
University of Helsinki
Haartmaninkatu 3
SF-00290 Helsinki 29
Finland

Table 4 *The average copper and zinc concentrations of human milk according to different authors*

Cu (mg/l)	Zn (mg/l)	No of samples	Stage of lactation	Reference
0.48	–	115	1st week	Munch Petersen 1950 (12)
–	5.3–3.6	?	*4th day*	Berfenstam 1952 (4)
–	0.65	?	6th month	
0.56	3.54	9–11	2nd–6th month	Belavady & Gopalan 1959 (3)
0.67–0.89	–	?	1st week after	Beck 1960 (2)
0.15–0.17	–	?	several months	
0.25	–	30	6th–7th day	Grebennikov et al 1961 (7)
1.34	–	15	1st week	Kleinbaum 1962 (11)
0.26	–	52	5th–6th month	
0.62	4.08	10	5th–8th day	Cavell & Widdowson 1964 (5)
0.24	1.34	22	?	Murthy & Rhea 1971 (13)
–	3.7	17[b]	2nd week	Jensen et al 1972 (10)
–	1.1	12[b]	18th week	
0.36	5.07	55[b]	5th day	Nassi et al 1974 (14)
0.29	3.24	?	15th day	
0.24	1.63	350	6th–12th week	Picciano & Guthrie 1976 (15)
0.39	2.95	96	4th–6th week	Anon 1977 (1)
0.60[c]	4.0[c]	229	2nd week–31st week	Present study
0.27	0.44[c]			

[a] The authors give ppm in ash
[b] Number of mothers
[c] Median value

hospital when the same mothers are to be followed up during the entire lactation period such a method is difficult to follow In the present study we have had to be satisfied with the second best but practical method the milk samples represent every feed during a period of 24 hours foremilk and hindmilk in equal proportions Furthermore the mothers took the samples by manual milking directly into the sampling bottles So we have tried to minimize both contamination and the effect of known variations in the components of milk

All the mothers used in this study were primipara healthy and well nourished The subjects volunteered for the study before breast feeding had begun The fact that the breast feeding of this group of mothers was successful is partly attributable to the continuous encouraging to carry on breast feeding and partly due to the 6 month maternity leave which is now granted to mothers in Finland

Both the copper and zinc concentrations of human milk are reported to diminish during lactation (17) The individual variations are said to be great (17) Recently Picciano & Guthrie demonstrated diurnal variations as well the copper and zinc levels seem to be higher in the morning than later in the same day (15) No significant difference has been found between the copper content of foremilk and hindmilk (12)

The average levels of trace element concentrations of human milk are given here using medians instead of means This is done because the distributions of values were found to be skewed Calculated mean values over estimate the actual average zinc and under estimate the copper content However the differences between the medians and the means are not great being at highest 10–15% divergent from the median values But if the variation of individual values is expressed by giving the standard deviation it overestimates the real variation because of the skewed distribution So we have expressed the variation by giving the total ranges the 25th and

Acta Pædiatr Scand 68 39–41 1979

OSTEOMYELITIS OF THE PUBIS

F HELDRICH and V HARRIS

From the Department of Pediatrics St Agnes Hospital Baltimore Maryland USA

ABSTRACT Heldrich F J and Harris V C (Department of Pediatrics St Agnes Hospital Baltimore Maryland USA) Osteomyelitis of the pubis Acta Paediatr Scand 67 39 1979 —Three cases of osteomyelitis of the pubis are presented and the clinical and laboratory features summarized A review of the literature revealed 49 cases and emphasizes the rarity of this location for acute primary osteomyelitis

KEY WORDS Osteomyelitis pubis childhood

Pelvic osteomyelitis has been documented as occurring in 2% to 11% of patients of all ages reported in eight large series (4 12 13 15 17 18) The pubic bone is involved least commonly of all of the bones of the pelvis we were able to find 49 cases including both children and adults of osteomyelitis involving the pubic bone reported from 1883–1978 (5 6 8 9 12 13 16 17) This paper reports the findings in three patients with acute primary osteomyelitis of the pubis The rarity of this entity as well as its clinical presentation prompted this report

CASE REPORTS

Case 1 M C a 6½ year-old white male admitted with limping and pain in the left hip of ten days duration prior and high fever with delirium one day prior to admission Four years and two years previously the child had experienced pain in the left hip lasting several days Radiographs were negative and no therapy was instituted Four weeks prior to this admission the patient again had pain in the left hip Physical examination and hip radiographs were negative Three weeks prior to admission lesions of erythema nodosum appeared on the lower extremities A throat culture was positive for group A beta hemolytic *Streptococci* and erythromycin 250 mg every six hours was given for ten days No history of trauma Examination revealed temperature 39 6 C pulse 128 beats per minute respiratory rate of 4 breaths per minute blood pressure 100/64 mmHg There was limitation of voluntary movement of the left hip with marked resistance to active internal rotation No point tenderness on palpation of the hip joint or trochanter Fullness was apparent in the left femoral triangle Special studies included Peripheral white cell count 23 200/mm³ 74% neutrophils 17% lymphocytes 6 monocytes 2 eosinophils 1 band Erythrocyte sedimentation rate 41 Blood cultures times 2 negative Radiographs revealed a radiolucent lesion in the inferior ramus of the left pubic bone Fifteen cc of pus were aspirated anterior and medial to the hip which grew *Staphylococcus aureus* An abscess outside the hip joint extending from the pubic bone was drained surgically Intravenous methicillin was given for four weeks followed by oral erythromycin for two weeks Four months later the patient was clinically well with radiologic improvement of the lesion in the pubic bone

Case 2 M Y a 9 year-old female was admitted with pain in the right hip of five days duration and a high fever for one day Two weeks prior to admission she had complained of pain in the right thigh Physical examination was normal at that time On admission temperature 40°C pulse 92 beats per minute respiratory rate 20 breaths per minute blood pressure 114/70 mmHg The patient limped on the right hip When prone the hip was held in external rotation and abduction Palpation and passive movements of the hip were not painful Laboratory data revealed Peripheral leucocyte count 17 400/mm with 66% neutrophils 13% lymphocytes 8% monocytes 13% bands Erythrocyte sedimentation rate 39 Initial radiographs of the hip pelvis femur and lumbosacral region were negative Blood cultures times 3 were negative Synovial fluid aspirated from the hip was sterile The child remained symptomatic and was started on intravenous methicillin On the seventh hospital day repeat radiographs revealed a lytic lesion in the inferior ramus of the right pubis Total therapy consisted of ten days of intravenous methicillin and six weeks of oral cloxacillin Nine months later the patient was doing well and radiographs revealed marked improvement (Fig 1)

Case 3 C J a 7 year-old white female was admitted with sore throat for three days high fever for two days and pain in the right hip one day prior to admission

day after admission respectively Shielding of the gonads in males during the X ray procedure may interfere with visualization of the pubic bone Radioisotopic delineation of osteomyelitis may be possible earlier than conventional radiographs but Case 3 demonstrated that accumulation of the radioisotope in the bladder urine may interfere with the visualization of the bones of the pelvis

Experience with three cases of acute primary osteomyelitis of the pubis has enabled us to summarize their salient features of presentation (Table 1)

Failure to detect osteomyelitis as the cause of illness may lead to inadequate therapy and thus predispose to chronic infection or other complications Management should be in accordance with accepted standards for osteomyelitis (2 7 10 14) Recovery of an organism preferably from the site of involvement should determine the choice of antibiotics Organisms recovered were penicillin resistant *Staphylococcus aureus* by tissue aspiration in Case 1 and a group A beta hemolytic *Streptococcus* by blood culture in Case 3 All patients responded to the antibiotics chosen and recovery was satisfactory in each instance

CONCLUSION

Osteomyelitis of the pubis while uncommon should be included in the differential diagnosis for patients presenting with symptoms suggestive of hip pathology Our observations from these three reported cases suggest the following findings to be helpful

1 Severe pain elicited on active motion of the hip but not on passive motion
2 Absence of radiologic confirmation of hip involvement (including soft tissue signs)
3 Normal synovial fluid on hip aspiration
4 Tenderness over the pubic area

REFERENCES

1 Aksby R L & Staheli L T Pyogenic infections of the sacroiliac joint in children (Radioisotope bone scanning as a diagnostic tool) *Clin Orthop 100* 96 1974

2 Chaklin V D Management of severe forms of haematogenous osteomyelitis of the pelvic bone *Ortop Travmatol Protez* 27 29 1966 (Russ)

3 Chung S M K & Borns P Acute osteomyelitis adjacent to the sacroiliac joint in children (Report of 2 cases) *J Bone Joint Surg 55A* 630 1973

4 Dich V Q Nelson J D & Haltalin K C Osteomyelitis of infants and children A review of 163 cases *Am J Dis Child 129* 1273 1975

5 Dinan J J Two cases of primary osteomyelitis of the pubic bone *Can Med Assoc J 41* 436 1939

6 Edwards M S Baker C J Granberry W M & Barrett F F Pelvic osteomyelitis in children *Pediatrics 61* 62 1978

7 Ferguson A B Jr Osteomyelitis in children *Clin Orthop 96* 51 1973

8 Fournier A Pauli A Ducoulombier H Cousin J & Huguet J Osteomyelitis of the pubic bone *J Sci Med Lille 85* 391 1967 (Fr)

9 Geist E S Osteomyelitis of pubic bone *JAMA 77* 1939 1921

10 Kulowski J Hicks E O & Steindler A Operative treatment of suppurative lesions of the bony pelvis *Am J Surg* 79 405 1950

11 Letts R M Afifi A & Sutherland J B Technitium bone scanning as an aid in the diagnosis of atypical osteomyelitis in children *Surg Gynecol Obstet 140* 899 1975

12 McWhorter G Osteomyelitis of the ischium and pubis *Surg Gynecol Obstet 49* 205 1929

13 Morgan A & Yates A K The diagnosis of acute osteomyelitis of the pelvis *Postgrad Med J 42* 74 1966

14 Nelson J D The bacterial etiology and antibacterial management of septic arthritis in infants and children *Pediatrics 50* 437 1972

15 Scott L Osteomyelitis of the pelvis *South Med J 20* 104 1927

16 Waldvogel F A Medoff G & Swartz M N Osteomyelitis A review of clinical features therapeutic considerations and unusual aspects (Part I) *N Engl J Med 282* 198 1976

17 Wilensky A O Osteomyelitis of the pelvic girdle *Arch Surg 37* 371 1938

18 Young F Acute osteomyelitis of the ilium *Surg Gynecol Obstet* 58 986 1934

Submitted Febr 25 1978
Accepted May 16 1978

(F J H) Department of Pediatrics
St Agnes Hospital
900 Caton Avenue
Baltimore
Maryland 21229
USA

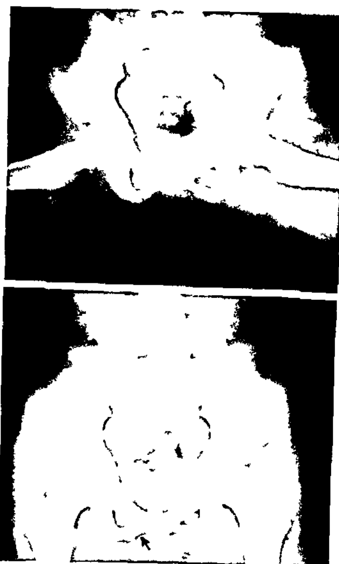


Fig. 1 Patient M Y (A) Lytic lesion in the inferior ramus of the right pubis (B) Follow up radiograph showing improvement 9 months later

Temperature was 40 4 C pulse 124 beats per minute respiratory rate 30 breaths per minute blood pressure 110/60 mmHg The patient limped on the right hip which was held externally rotated flexed and abducted with marked limitation of active movement There was tenderness on palpation just lateral to the symphysis pubis on the right Laboratory studies were Peripheral leucocyte count 22 000/mm³ with 57% neutrophils 3% lymphocytes 1% monocytes 39% bands Erythrocyte sedimentation rate 44 Radiographs of the hip and pelvis were negative Accumulation of dye in the urinary bladder on technetium scan obscured the pelvic girdle Blood culture grew beta hemolytic *Streptococci* group A Hip aspiration was negative The child was started on intravenous methicillin and subsequently changed to penicillin G On the 22nd day a radiograph revealed a lytic lesion in the inferior ramus of the right pubic bone The patient received six weeks of intravenous antibiotics and four months later was doing well with demonstrated radiologic improvement

Table 1 *Summary of findings*

	Case 1	Case 2	Case 3
History			
Fever	+	+	+
Hip pain	+	+	+
Pubic pain	−	−	−
Trauma	0	0	0
Physical findings			
Hip pain			
Active motion	+	+	+
Passive motion	−	−	+
Pubic tenderness	−	−	+
Lab data			
Leucocytosis	+	+	+
Elevated sed rate	+	+	+
X Rays			
Hip	−	−	−
Pubis	+	+	+
Cultures			
Blood	−	−	+
Pubis	+	0	0
Soft tissue	+	0	0
Hip joint	−	−	−

DISCUSSION

The initial diagnosis in all three cases on admission was septic arthritis of the hip This condition requires early recognition and prompt surgical drainage to minimize permanent damage to the hip joint Noteworthy in our cases is the fact that passive motion of the hip was reasonably well tolerated whereas active motion particularly when weight bearing was not In such instances osteomyelitis involving the metaphysis of the femur would be the most likely site of involvement But as our cases indicate the bones of the pelvic girdle may also be the site of pathology In Case 3 our house staff now alerted to this possibility found tenderness over the pubis and made the provisional diagnosis

Aids to the diagnosis of osteomyelitis include radiographs and radionucleotide scans but these have limitations also (1 11) Radiologic evidence of osteomyelitis is positive only 10 to 14 days or more after onset In two of our cases the radiographs did not become positive until the seventh and twenty second

day after admission respectively Shielding of the gonads in males during the X ray procedure may interfere with visualization of the pubic bone Radioisotopic delineation of osteomyelitis may be possible earlier than conventional radiographs but Case 3 demonstrated that accumulation of the radioisotope in the bladder urine may interfere with the visualization of the bones of the pelvis

Experience with three cases of acute primary osteomyelitis of the pubis has enabled us to summarize their salient features of presentation (Table 1)

Failure to detect osteomyelitis as the cause of illness may lead to inadequate therapy and thus predispose to chronic infection or other complications Management should be in accordance with accepted standards for osteomyelitis (2 7 10 14) Recovery of an organism preferably from the site of involvement should determine the choice of antibiotics Organisms recovered were penicillin resistant *Staphylococcus aureus* by tissue aspiration in Case 1 and a group A beta hemolytic *Streptococcus* by blood culture in Case 3 All patients responded to the antibiotics chosen and recovery was satisfactory in each instance

CONCLUSION

Osteomyelitis of the pubis while uncommon should be included in the differential diagnosis for patients presenting with symptoms suggestive of hip pathology Our observations from these three reported cases suggest the following findings to be helpful

1 Severe pain elicited on active motion of the hip but not on passive motion

2 Absence of radiologic confirmation of hip involvement (including soft tissue signs)

3 Normal synovial fluid on hip aspiration

4 Tenderness over the pubic area

REFERENCES

1 Aksby R L & Staheli L T Pyogenic infections of the sacroiliac joint in children (Radioisotope bone scanning as a diagnostic tool) *Clin Orthop 100* 96 1974

2 Chaklin V D Management of severe forms of haematogenous osteomyelitis of the pelvic bone *Ortop Travmatol Prote* 27 29 1966 (Russ)

3 Chung S M K & Borns P Acute osteomyelitis adjacent to the sacroiliac joint in children (Report of ? cases) *J Bone Joint Surg 55A* 630 1973

4 Dich V Q Nelson J D & Haltalin K C Osteomyelitis of infants and children A review of 163 cases *Am J Dis Child 129* 1?73 1975

5 Dinan J J Two cases of primary osteomyelitis of the pubic bone *Can Med Assoc J 41* 436 1939

6 Edwards M S Baker C J Granberry W M & Barrett F F Pelvic osteomyelitis in children *Pediatrics 61* 6? 1978

7 Ferguson A B Jr Osteomyelitis in children *Clin Orthop 96* 51 1973

8 Fournier A Pauli A Ducoulombier H Cousin J & Huguet J Osteomyelitis of the pubic bone *J Sci Med Lille 85* 391 1967 (Fr)

9 Geist E S Osteomyelitis of pubic bone *JAMA 77* 1939 19?1

10 Kulowski J Hicks E O & Steindler A Operative treatment of suppurative lesions of the bony pelvis *Am J Surg 79* 405 1950

11 Letts R M Afifi A & Sutherland J B Technetium bone scanning as an aid in the diagnosis of atypical osteomyelitis in children *Surg Gynecol Obstet 140* 899 1975

12 McWhorter G Osteomyelitis of the ischium and pubis *Surg Gynecol Obstet 49* ?05 19?9

13 Morgan A & Yates A K The diagnosis of acute osteomyelitis of the pelvis *Postgrad Med J 42* 74 1966

14 Nelson J D The bacterial etiology and antibacterial management of septic arthritis in infants and children *Pediatrics 50* 437 197?

15 Scott L Osteomyelitis of the pelvis *South Med J 20* 104 19?7

16 Waldvogel F A Medoff G & Swartz M N Osteomyelitis A review of clinical features therapeutic considerations and unusual aspects (Part I) *N Engl J Med 282* 198 1976

17 Wilensky A O Osteomyelitis of the pelvic girdle *Arch Surg 37* 371 1938

18 Young F Acute osteomyelitis of the ilium *Surg Gynecol Obstet 58* 986 1934

Submitted Febr 25 1978
Accepted May 16 1978

(F J H) Department of Pediatrics
St Agnes Hospital
900 Caton Avenue
Baltimore
Maryland 21??9
USA

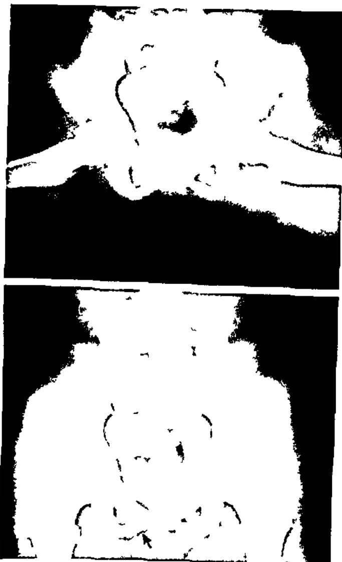


Fig 1 Patient M Y (A) Lytic lesion in the inferior ramus of the right pubis (B) Follow up radiograph showing improvement 9 months later

Temperature was 40 4 C pulse 124 beats per minute respiratory rate 30 breaths per minute blood pressure 110/60 mmHg The patient limped on the right hip which was held externally rotated flexed and abducted with marked limitation of active movement There was tenderness on palpation just lateral to the symphysis pubis on the right Laboratory studies were Peripheral leucocyte count 22000/mm³ with 57% neutrophils 3% lymphocytes 1% monocytes 39% bands Erythrocyte sedimentation rate 44 Radiographs of the hip and pelvis were negative Accumulation of dye in the urinary bladder on technetium scan obscured the pelvic girdle Blood culture grew beta hemolytic *Streptococci* group A Hip aspiration was negative The child was started on intravenous methicillin and subsequently changed to penicillin G On the 22nd day a radiograph revealed a lytic lesion in the inferior ramus of the right pubic bone The patient received six weeks of intravenous antibiotics and four months later was doing well with demonstrated radiologic improvement

Table 1 *Summary of findings*

	Case 1	Case 2	Case 3
History			
Fever	+	+	+
Hip pain	+	+	+
Pubic pain	–	–	–
Trauma	0	0	0
Physical findings			
Hip pain			
Active motion	+	+	+
Passive motion	–	–	+
Pubic tenderness	–	–	+
Lab data			
Leucocytosis	+	+	+
Elevated sed rate	+	+	+
X Rays			
Hip	–	–	–
Pubis	+	+	+
Cultures			
Blood	–	–	+
Pubis	+	0	0
Soft tissue	+	0	0
Hip joint	–	–	–

DISCUSSION

The initial diagnosis in all three cases on admission was septic arthritis of the hip This condition requires early recognition and prompt surgical drainage to minimize permanent damage to the hip joint Noteworthy in our cases is the fact that passive motion of the hip was reasonably well tolerated whereas active motion particularly when weight bearing was not In such instances osteomyelitis involving the metaphysis of the femur would be the most likely site of involvement But as our cases indicate the bones of the pelvic girdle may also be the site of pathology In Case 3 our house staff now alerted to this possibility found tenderness over the pubis and made the provisional diagnosis

Aids to the diagnosis of osteomyelitis include radiographs and radionucleotide scans but these have limitations also (1 11) Radiologic evidence of osteomyelitis is positive only 10 to 14 days or more after onset In two of our cases the radiographs did not become positive until the seventh and twenty second

day after admission respectively Shielding of the gonads in males during the X ray procedure may interfere with visualization of the pubic bone Radioisotopic delineation of osteomyelitis may be possible earlier than conventional radiographs but Case 3 demonstrated that accumulation of the radioisotope in the bladder urine may interfere with the visualization of the bones of the pelvis

Experience with three cases of acute primary osteomyelitis of the pubis has enabled us to summarize their salient features of presentation (Table 1)

Failure to detect osteomyelitis as the cause of illness may lead to inadequate therapy and thus predispose to chronic infection or other complications Management should be in accordance with accepted standards for osteomyelitis (2 7 10 14) Recovery of an organism preferably from the site of involvement should determine the choice of antibiotics Organisms recovered were penicillin resistant *Staphylococcus aureus* by tissue aspiration in Case 1 and a group A beta hemolytic *Streptococcus* by blood culture in Case 3 All patients responded to the antibiotics chosen and recovery was satisfactory in each instance

CONCLUSION

Osteomyelitis of the pubis while uncommon should be included in the differential diagnosis for patients presenting with symptoms suggestive of hip pathology Our observations from these three reported cases suggest the following findings to be helpful

1 Severe pain elicited on active motion of the hip but not on passive motion

2 Absence of radiologic confirmation of hip involvement (including soft tissue signs)

3 Normal synovial fluid on hip aspiration

4 Tenderness over the pubic area

REFERENCES

1 Aksby R L & Staheli L T Pyogenic infections of the sacroiliac joint in children (Radioisotope bone scanning as a diagnostic tool) *Clin Orthop 100* 96 1974

2 Chaklin V D Management of severe forms of haematogenous osteomyelitis of the pelvic bone *Ortop Travmatol Protez* 27 29 1966 (Russ)

3 Chung S M K & Borns P Acute osteomyelitis adjacent to the sacroiliac joint in children (Report of 2 cases) *J Bone Joint Surg 55A* 630 1973

4 Dich V Q Nelson J D & Haltalin K C Osteomyelitis of infants and children A review of 163 cases *Am J Dis Child 129* 1273 1975

5 Dinan J J Two cases of primary osteomyelitis of the pubic bone *Can Med Assoc J 41* 436 1939

6 Edwards M S Baker C J Granberry W M & Barrett F F Pelvic osteomyelitis in children *Pediatrics 61* 6 1978

7 Ferguson A B Jr Osteomyelitis in children *Clin Orthop 96* 51 1973

8 Fournier A Pauli A Ducoulombier H Cousin J & Huguet J Osteomyelitis of the pubic bone *J Sci Med Lille 85* 391 1967 (Fr)

9 Geist E S Osteomyelitis of pubic bone *JAMA 77* 1939 1921

10 Kulowski J Hicks E O & Steindler A Operative treatment of suppurative lesions of the bony pelvis *Am J Surg 79* 405 1950

11 Letts R M Afifi A & Sutherland J B Technetium bone scanning as an aid in the diagnosis of atypical osteomyelitis in children *Surg Gynecol Obstet 140* 899 1975

12 McWhorter G Osteomyelitis of the ischium and pubis *Surg Gynecol Obstet 49* 205 1929

13 Morgan A & Yates A K The diagnosis of acute osteomyelitis of the pelvis *Postgrad Med J 42* 74 1966

14 Nelson J D The bacterial etiology and antibacterial management of septic arthritis in infants and children *Pediatrics 50* 437 1972

15 Scott L Osteomyelitis of the pelvis *South Med J 20* 104 1927

16 Waldvogel F A Medoff G & Swartz M N Osteomyelitis A review of clinical features therapeutic considerations and unusual aspects (Part I) *N Engl J Med 282* 198 1976

17 Wilensky A O Osteomyelitis of the pelvic girdle *Arch Surg 37* 371 1938

18 Young F Acute osteomyelitis of the ilium *Surg Gynecol Obstet 58* 986 1934

Submitted Febr 25 1978
Accepted May 16 1978

(F J H) Department of Pediatrics
St Agnes Hospital
900 Caton Avenue
Baltimore
Maryland 21229
USA

Acta Pædiatr Scand 68 43–48 1979

COMPARISONS BETWEEN SERUM CONCENTRATIONS OF THYROXINE AND THYROXINE BINDING PROTEINS IN SAMPLES SIMULTANEOUSLY OBTAINED FROM CAPILLARY PERIPHERAL VEIN CENTRAL VEIN AND AORTA IN NEWBORN INFANTS

B BROCK JACOBSEN and B PEITERSEN

From the University Clinic of Paediatric Children s Hospital Fuglebakken Copenhagen Denmark

ABSTRACT Jacobsen B B and Peitersen B (University Clinic of Paediatrics Children s Hospital Fuglebakken Copenhagen Denmark) Comparisons between serum concentrations of thyroxine and thyroxine binding proteins in samples simultaneously obtained from capillary peripheral vein central vein and aorta in newborn infants Acta Paediatr Scand 68 43 1979 —A total number of 40 newborn infants with various maturity were studied 13 babies without perinatal events 19 infants recovered from transient diseases 6 infants with idiopathic respiratory distress syndrome and 2 infants with asphyxia indicating artificial ventilation Comparisons were performed between serum concentrations of thyroxine (T) thyroxine-binding globulin (TBG) prealbumin (TBPA) and albumin (Alb) in capillary versus peripheral vein aorta versus central vein and finally in peripheral versus central veins In healthy infants serum T_4 concentrations in capillary blood and peripheral vein did not differ significantly Although serum concentrations of thyroid hormone-binding proteins tended to be increased in aortic compared to central venous specimens no statistically significant differences appeared In infants in good clinical conditions serum T TBG TBPA and Alb levels were 6–8% higher in peripheral than in central veins possibly primarily due to a hemoconcentrating effect of venous stasis Therefore in evaluation of the thyroid variables in newborn infants the technique of blood sampling must be considered In most infants with idiopathic respiratory distress syndrome and in one asphyxiated baby a remarkable tendency to a low serum TBG and T concentration in peripheral compared to central vein samples were observed

KEY WORDS thyroxine thyroxine binding globulin prealbumin albumin idiopathic respiratory distress syndrome neonates

Venous compression by arm tourniquet rises the filtration pressure across the capillary wall (8) and may increase total serum concentrations of thyroid hormones (16) Recently we demonstrated in adults that even shortterm venous stasis increases serum thyroxine and thyroxine binding protein levels by 7–11% (12) The capillary blood flow and the transcapillary escape of fluid and proteins are much higher in newborns than in adults (1 17 18 21 27) It has not yet been possible for technical reasons to perform similar investigations of the influence of venous stasis in newborn infants

In newborn babies with low birth weights or idiopathic respiratory distress syndrome (IRDS) low serum thyroxine and thyroxine binding protein concentrations have been observed (3 11 13 14 23) These findings—also of relevance in screening for congenital hypothyroidism (7)—have not been fully explained

The present study was conducted in neonates with various maturity to compare serum concentrations of thyroxine and thyroxine

Table 1 *Clinical data in 40 newborn infants aged 1 to 4 days*
The groups of infants—see text

Group of infants	Birth weights (g)	Gestational age (weeks)	No of infants		
			Fullterm	Premature	Small for date
I	2 960 (1 890–3 960)	38 (33–41)	7	3	3
II	2 450 (1 520–4 100)	39 (31–40)	7	4	8
III	2 585 (1 050–3 650)	35 (28–43)	3	4	1

binding proteins in samples obtained simultaneously from different parts of the vascular system capillaries peripheral veins after application of arm tourniquet and from central veins and aorta

MATERIAL AND METHODS

A total number of 40 newborn infants aged 1 to 4 days were included in the study All were euthyroid with serum thyrotropin concentrations within normal range (11) Maturity and gestational age were assessed as previously reported (10) From clinical conditions the infants were divided in three groups (Table 1)

Group I were healthy infants without perinatal asphyxia

Group II were infants in clinical good condition with normal acid base status recovered from transient hypoglycemic hypocalcemic or respiratory problems which had indicated umbilical catheterization

Group III consisted of six infants with idiopathic respiratory distress syndrome and one premature and one postmature baby with severe asphyxia indicating intubation artificial ventilation and application of umbilical catheters All infants had stabilised circulation and normal body temperature when the study was performed

Argyle® umbilical catheters fr size 3½ or 5 were used The artery catheters were placed with the tip in the descendent aorta at the level of 10th to 12th thoracal vertebra The umbilical vein catheter tips were usually placed in the umbilical vein but in two infants the tip was in the portal sinus Only in one infant the catheter tip was situated in the right atrium No complications in relation to the catheterization were seen Parents were informed and consent obtained At least two blood samples were obtained simultaneously from each infant The amount of blood taken from each infant did not exceed 5–6 ml *Capillary* blood was drawn after heel prick and *peripheral venous samples* were obtained from a cubital vein after application of a tourniquet of rubber on the upper arm Blood samples from the *central venous system* and *aorta* were taken from the umbilical catheters after withdrawing 2 ml blood prior to each sampling This sample was reinjected after the test sample had been obtained Blood was centrifuged and serum frozen at −20 until analyses were performed All analyses were performed in duplicate

Serum *total thyroxine* (T_4) was measured using a competitive binding microtechnique as previously reported (10) The intraassay coefficient of variation was 4% in the range of 77–129 nmol/l and 6–10% outside that range *Serum thyroxine binding globulin (TBG)* was measured using rocket immunoelectrophoresis and autoradiography (14) The intraassay coefficient of variation was 4% *Serum prealbumin (TBPA)* and *albumin (Alb)* were determined by immunoelectrophoresis as reported elsewhere (14) The intraassay coefficients of variation for both protein assays were 3% The serum concentrations of thyroid hormone binding proteins were presented in terms of those in a reference serum pool from male adults and stated to 100 arbitrary units per liter Using routine laboratory methods the absolute serum TBG TBPA and Alb concentrations in the reference serum are 9 3 mg/l 97 mg/l and 43 000 mg/l respectively (14) Normal ranges of serum T_4 TBG TBPA and Alb in newborn infants have been reported previously (11 14)

In the statistical analyses the Wilcoxon s test for pair differences the Spearman rank correlation coefficient R and the chi square (χ^2) test with Yate s correction were used (25)

RESULTS

The investigations were tripartite Only serum concentrations in simultaneously obtained blood samples were compared

Serum T_4 in capillary versus peripheral venous samples

In 13 healthy infants (group I) with various maturity a comparison was performed between serum T_4 concentrations in capillary versus in peripheral venous blood In capil

Table 2 *Serum thyroxine (T_4) thyroxine binding globulin (TBG) prealbumin (TBPA) and albumin (Alb) concentrations in blood samples obtained simultaneously from aorta and central venous system*

The groups of infants—see text

Groups of infants		Serum T (nmol/l)		Serum TBG (arb u /l)		Serum TBPA (arb u /l)		Serum Alb (arb u /l)	
		Aorta	Central vein	Aorta	Central vein	Aorta	Central vein	Aorta	Central vein
II	Median	15	?14	191	168	?8	27	77	80
	Range	(111–?75)	(116–?81)	(111–?34)	(113–?39)	(25–37)	(23–37)	(65–86)	(55–83)
	No	8		6		6		7	
		$p>0\ 1$		$p>0\ 1$		$p>0\ 1$		$p>0\ 1$	
III	Median	1?2	1?6	138	133	?3	??	78	74
	Range	(64–134)	(6?–14?)	(10_– 06)	(94– 05)	(17–30)	(17–28)	(64–95)	(60–91)
	No	6		6		6		6	
		$p>0\ 1$		$p>0\ 1$		$p>0\ 1$		$p>0\ 1$	

p is the level of significance for the Wilcoxon test for pair differences

lary blood serum T_4 ranged 93–208 nmol/l (median 167 nmol/l) In *venous* samples serum T_4 ranged 93–212 nmol/l (median 154 nmol/l) which did not differ significantly from those in capillary blood ($p>0\ 1$) The serum T_4 concentrations but not the individual differences between capillary and venous concentrations were influenced by maturity and postnatal age Measurement of thyroxine binding proteins was not performed because an adequate amount of serum was not available

Serum T_4 TBG TBPA and Alb in samples from umbilical vessel catheters

In 14 infants from group II and III blood samples were drawn simultaneously from aorta and the central venous system Table 2 shows median and range of the serum T_4 TBG TBPA and Alb concentrations In the four infants with idiopathic respiratory distress syndrome serum T_4 and TBG levels were lower than those of other neonates whereas in the two asphyxiated infants serum T_4 and TBG

Table 3 *Serum thyroxine (T_4) thyroxine binding globulin (TBG) prealbumin (TBPA) and albumin (Alb) concentrations in specimens from peripheral (P V) and central veins (C V) obtained simultaneously*

The groups of infants—see text

Groups of infants		Serum T (nmol/l)		Serum TBG (arb u /l)		Serum TBPA (arb u /l)		Serum Alb (arb u /l)	
		P V	C V	P V	C V	P V	C V	P V	C V
II	Median	184	175	164	153	?6	?4	76	75
	Range	(85–317)	((80–?81)	(1?1–?34)	(119– ??)	(17–35)	(15–3?)	(49–101)	(55–88)
	No	19		16		17		18	
		$p<0\ 05$		$p<0\ 01$		$p<0\ 01$		$p<0\ 01$	
III	Median	108	111	13	136	?6	4	79	74
	Range	(75–1?6)	(6 –14?)	(81–?08)	(85–?05)	(19– 9)	(17–?8)	(63–98)	(69–91)
	No	7		7		8		7	
		$p>0\ 1$		$p>0\ 1$		$p>0\ 1$		$p>0\ 1$	

p is the level of significance for the Wilcoxon test for pair differences

were comparable to those in healthy infants with similar birth weights Although serum protein concentrations tended to be higher in aortic than in venous samples no statistically significant differences were observed The individual differences between serum concentrations in aorta and central veins were not related to maturity birth weight or disease

Serum T_4 TBG TBPA and Alb in samples from peripheral versus central veins

In 22 infants a comparison between serum T_4 TBG TBPA and Alb level in peripheral versus central veins was performed Furthermore in 5 infants serum T_4 and T_4 binding protein concentrations in peripheral veins were compared with those in aorta and the results were included in the calculations (Table 3) This does not influence the conclusions because serum concentrations in aorta and central veins are comparable

Table 3 shows the median and range values of serum T_4 TBG TBPA and Alb in 19 infants in a good clinical condition (group II) and in 6 infants with IRDS and 2 asphyxiated babies (group III) In group II infants serum concentrations of T_4 and T_4 binding proteins were significantly higher in peripheral than in central veins (and aorta) The median increases were 6–8% and a statistically significant correlation was found between increases in serum T_4 and serum TBG concentrations (R 0 51 $p<0$ 05)

In infants in group III serum TBPA and serum Alb levels were also higher in peripheral than in central veins (although not statistically significant) Serum T_4 and TBG in contrast tended to be *lower* in the peripheral vein In fact serum TBG concentrations in peripheral veins were significantly more often reduced in infants with IRDS than in the other infants studied (χ 4 01 $p<0$ 05) whereas this was not statistically significant concerning serum T_4 (χ^2 1 08 $p>0$ 1)

Median values of serum T_4 and TBG concentrations (Table 3) in both peripheral and central veins were lower in group III than in group II infants

DISCUSSION

Previous investigations have demonstrated a reasonably good correlation between T_4 concentrations in dried capillary blood (eluated from filter paper recovery 74–95%) and in whole blood or plasma (4 20 28) but it was noticed that in infants capillary serum T_4 samples may be variably unreliable (28) In the present study however capillary blood (after heel prick) was sampled in glasses immediately centrifuged and serum frozen until analysis was performed Using that technique serum T_4 concentrations in capillary and peripheral venous blood did not differ significantly neither in fullterm nor in low birth weight newborns The discrepancy between previous and present studies seems to be due to different methodology

The present finding of comparable serum T_4 TBG and Alb concentrations in samples from umbilical artery and vein catheters seems of some importance Ewerbeck & Levens (6) and Tulzer (26) measured a higher serum Alb and a lower alfa globulin concentration in umbilical artery than in umbilical vein specimens Later Rose et al (24) have reported that protein bound iodine concentrations in umbilical vein and artery specimens were of the same magnitude which does agree with our results These earlier investigations were performed using samples from a clamped umbilical cord at the delivery which might involve measurement of placental proteins Determinations of serum T_4 and T_4 binding protein concentrations in the central arterial and venous system of human newborns have not previously been reported The samples from the central venous system consisted mainly of blood from the portal vein Due to a sphincter function of ductus venosus (15) probably only a small amount of blood was coming from the inferior caval vein The tendency towards higher serum protein concentrations in aorta compared

o central veins may be due to the hepatic protein synthesis

The most important observation in the present study may be the finding in group II infants of higher serum concentrations of T_4 and T_4 binding proteins in peripheral vein specimens compared to those in blood from the central vascular system obtained simultaneously For the infants in a clinical good condition serum concentrations of T_4 TBG TBPA and Alb were 6–8% higher in peripheral vein samples and a statistically significant correlation was found between the increase in serum T_4 and in serum TBG These findings might be explained by a *hemoconcentrating effect induced by peripheral venous stasis*

In adults peripheral venous stasis causes a transudation of proteinfree solute from the circulation with a resultant rise in plasma protein concentrations (2 8) Circulating thyroid hormones are almost totally bound to serum protein (22) and it is known that in adults venous compression by arm tourniquet may increase serum concentrations of thyroid hormones by 7–15% (16) Recently we demonstrated that such increases in serum T_4 as well as in serum TBG TBPA and Alb concentrations occur after *shortterm* (2–3 min) venous stasis (12) We have not been able to obtain a sufficient amount of blood in similar investigations in newborn infants

During the early neonatal period of life the transcapillary movements of fluids (1 17 27) and proteins are increased compared to later in infancy In fact the transcapillary escape rate of Alb is 3–4 times higher in the newborn mature infants than in adults (18 21) which might also apply to the other T_4 binding proteins (9) The transcapillary escape and blood flow is even higher in premature than in mature newborns (1 17) These observations however were not reflected in the present results possibly because recirculation of fluids and proteins to the vascular system is also increased during the neonatal period

In infants with IRDS or asphyxia the peripheral blood flow is reduced and the vascular resistance increased (1 17) possibly due to increased catecholamine release (19) Furthermore changes in blood pressure were probable in group III patients due to the intubation and artificial ventilation which might contribute to the explanation of the individual differences observed Our findings of lower median serum T_4 and TBG concentrations in infants with IRDS than in the other infants with comparable birth weights are in accordance with previous investigations (3 13 23) Serum TBG was more often *reduced* in peripheral veins as compared to central venous specimens in infants with IRDS than in other infants Further confirmations are clearly needed before any conclusions might be drawn

Previous investigations have demonstrated the significance of gestational age birth weight postnatal age and diseases for serum levels of T_4 TBG TBPA and Alb in newborn infants (3 5 11 13 14 23) The present study indicates that the site of blood sampling must also be considered

ACKNOWLEDGEMENTS

The technical assistance of Mrs K Jorgensen Mrs A Wilson and the staff of the X ray department is gratefully acknowledged We are grateful to Professor Dr Med H J Andersen for his help and encouragement Thanks also to Miss A Jacobsen for valuable secretarial help

The study was supported by The Danish Medical Research Council and The Dagmar Marshall Foundation

REFERENCES

1 Celander O Studies of the peripheral circulation *In* D E Cassels (ed) *The heart and circulation in the newborn infant* Grune & Stratton New York 1966 p 98

2 Christiansen C Næstoft J Hvidberg E Larsen N E & Petersen B An easy procedure for in vivo estimation of protein binding and correction of elevated serum values induced by venous stasis *Clin Chim Acta* 62 65 1975

3 Cuestas R A Lindall A & Engel R R Low thyroid hormones and respiratory distress syndrome of the newborn Studies on cord blood *N Engl J Med* 295 298 1976

4 Dussault J H & Laberge C Dosage de la thyroxine par methode radio-immunologique dans l eluat de

were comparable to those in healthy infants with similar birth weights Although serum protein concentrations tended to be higher in aortic than in venous samples no statistically significant differences were observed The individual differences between serum concentrations in aorta and central veins were not related to maturity birth weight or disease

Serum T_4 TBG TBPA and Alb in samples from peripheral versus central veins

In 22 infants a comparison between serum T_4 TBG TBPA and Alb level in peripheral versus central veins was performed Furthermore in 5 infants serum T_4 and T_4 binding protein concentrations in peripheral veins were compared with those in aorta and the results were included in the calculations (Table 3) This does not influence the conclusions because serum concentrations in aorta and central veins are comparable

Table 3 shows the median and range values of serum T_4 TBG TBPA and Alb in 19 infants in a good clinical condition (group II) and in 6 infants with IRDS and 2 asphyxiated babies (group III) In group II infants serum concentrations of T_4 and T_4 binding proteins were significantly higher in peripheral than in central veins (and aorta) The median increases were 6–8% and a statistically significant correlation was found between increases in serum T_4 and serum TBG concentrations (R 0 51 $p<0$ 05)

In infants in group III serum TBPA and serum Alb levels were also higher in peripheral than in central veins (although not statistically significant) Serum T_4 and TBG in contrast tended to be *lower* in the peripheral vein In fact serum TBG concentrations in peripheral veins were significantly more often reduced in infants with IRDS than in the other infants studied (χ^2 4 01 $p<0$ 05) whereas this was not statistically significant concerning serum T_4 (χ 1 08 $p>0$ 1)

Median values of serum T_4 and TBG concentrations (Table 3) in both peripheral and central veins were lower in group III than in group II infants

DISCUSSION

Previous investigations have demonstrated a reasonably good correlation between T_4 concentrations in dried capillary blood (eluated from filter paper recovery 74–95%) and in whole blood or plasma (4 20 28) but it was noticed that in infants capillary serum T_4 samples may be variably unreliable (28) In the present study however capillary blood (after heel prick) was sampled in glasses immediately centrifuged and serum frozen until analysis was performed Using that technique serum T_4 concentrations in capillary and peripheral venous blood did not differ significantly neither in fullterm nor in low birth weight newborns The discrepancy between previous and present studies seems to be due to different methodology

The present finding of comparable serum T_4 TBG and Alb concentrations in samples from umbilical artery and vein catheters seems of some importance Ewerbeck & Levens (6) and Tulzer (26) measured a higher serum Alb and a lower alfa globulin concentration in umbilical artery than in umbilical vein specimens Later Rose et al (24) have reported that protein bound iodine concentrations in umbilical vein and artery specimens were of the same magnitude which does agree with our results These earlier investigations were performed using samples from a clamped umbilical cord at the delivery which might involve measurement of placental proteins Determinations of serum T_4 and T_4 binding protein concentrations in the central arterial and venous system of human newborns have not previously been reported The samples from the central venous system consisted mainly of blood from the portal vein Due to a sphincter function of ductus venosus (15) probably only a small amount of blood was coming from the inferior caval vein The tendency towards higher serum protein concentrations in aorta compared

Acta Pædiatr Scand 68 49–55 1979

SERUM CONCENTRATIONS OF THYROXINE BINDING GLOBULIN PREALBUMIN AND ALBUMIN IN HEALTHY FULLTERM SMALL FOR GESTATIONAL AGE AND PRETERM NEWBORN INFANTS

B BROCK JACOBSEN B PEITERSEN H J ANDERSEN and L HUMMER

From the University Clinic of Paediatrics Children s Hospital Fuglebakken and from the Department of Nuclear Medicine Rigshospitalet Copenhagen Denmark

ABSTRACT Jacobsen B B Peitersen B Andersen H J and Hummer L (The University Clinic of Paediatrics Children s Hospital Fuglebakken and the Department of Nuclear Medicine Rigshospitalet Copenhagen Denmark) Serum concentrations of thyroxine binding globulin prealbumin and albumin in healthy fullterm small for-gestational age and preterm newborn infants Acta Paediatr Scand 68 49 1979 —Simultaneous serum concentrations of thyroxine-binding globulin (TBG) prealbumin (TBPA) and albumin (Alb) were measured in 130 fullterm 32 small for-gestational age and 25 preterm infants during their first six days of life In all infants serum concentrations of TBG were higher and serum TBPA and Alb were lower than in male adults Even higher serum TBG levels wer found in the mothers There was no correlation between serum concentrations in paired maternal and cord sera In infants with birth weights appropriate for gestation serum TBG TBPA and Alb concentrations increased progressively with gestational age In small for-gestational age infants born at term serum concentrations of TBG and Alb were lower than those in fullterm but higher than those in premature newborns Serum TBPA in small for-gestational age babies was evne lower than seen in prematures A positive correlation was found between thyroid hormones and TBG concentrations not between serum TBPA and thyroid hormones The ratios between serum concentration of thyroid hormones and proteins might indicate that more thyroid hormonebinding sites are occupied in fullterm than in low birth weight newborns However the main reason for the different serum levels of thyroid hormones in fullterm small for-gestational age and preterm babies is probably the various serum TBG concentrations demonstrated in these infants

KEY WORDS Thyroxine binding globulin prealbumin albumin SGA infants preterm infants

Thyroid hormones in human serum are mainly bound to thyroxine binding globulin (TBG) prealbumin (TBPA) and albumin (Alb) (3 14 23) It has been shown that serum thyroxine concentration in fetuses (7) and in cord blood (20) increases with gestational age This relationship seems—at least in part—to be caused by variation in serum TBG binding capacity (1 7) However Oddie et al (20) did not find a correlation between gestational age and TBG concentration in cord sera from infants of 30 to 45 weeks gestation No data on any small for gestational age infants were given

Recently we reported that serum concentrations of thyroxine (T_4) and triiodothyronine (T_3) are lower in small for gestational age (SGA) babies born at term than in mature full term (FT) newborns even lower serum concentrations were seen i preterm (PT) infants with birth weights appropriate for gestation (12)

The present investigations were undertaken to measure the serum concentrations of TBG TBPA and Alb in healthy newborn infants of various maturity and gestational age and to study the relationship between the protein and thyroid hormone concentrations in these babies

sang séché Nouvelle méthode de depistage de l hypothyroidie néonatale *Union Med Canada 10?* 2062 1973

5 Erenberg A Phelps D L Lam R & Fisher D A Total and free thyroid hormone concentrations in the neonatal period *Pediatrics 53* 211 1974

6 Ewerbeck V H & Levens H E Uber die Bildung der Serumeiweisskörper in der Placenta sowie ihren Beitrag und Fettstoffwechsel des Feten *Monatsschr Kinderheilkd 99* 297 1951

7 Fisher D A Burrow G N Dussault J H Hollingsworth D R Larsen P R Man E B & Walfish P G Recommendations for screening programs for congenital hypothyroidism Report of a committee of the American Thyroid Association *J Pediatr 89* 692 1976

8 Gerbrandy J Leewen A M V Hellendoorn H B A & Vries L A The binding between electrolytes and serum proteins calculated from and in vivo filtration method *Clin Sci 19* 181 1960

9 Irvine C H G & Simpson Morgan M W Relative rates of transcapillary movement of free thyroxine protein bound thyroxine thyroxine binding proteins and albumin *J Clin Invest 54* 156 1974

10 Jacobsen B B Andersen H Dige Petersen H & Hummer L Pituitary thyroid responsiveness to thyrotropin releasing hormone in preterm and small for gestational age newborns *Acta Paediatr Scand 66* 541 1977

11 Jacobsen B B Andersen H J Peitersen B Dige Petersen H & Hummer L Serum levels of thyrotropin thyroxine and triiodothyronine in fullterm small for gestational age and preterm newborn babies *Acta Paediatr Scand 66 681* 1977

12 Jacobsen B B Petersen B & Andersen H Changes in serum levels of thyroxine and thyroxine binding proteins (TBG TBPA Albumin) induced by venous stasis *Acta Endocrinol (Kbh) 85* 39 1977

13 Jacobsen B B Peitersen B & Andersen H J Serum concentrations of thyroid hormone and thyroid hormone binding proteins in infants with respiratory distress syndrome *16th annual meeting of European Society for Pediatric Endocrinology* Cambridge England 1977

14 Jacobsen B B Peitersen B Andersen H J & Hummer L Serum concentrations of thyroxine binding globulin prealbumin and albumin in healthy fullterm small for gestational age and preterm newborn infants *Acta Paediatr Scand* 1978 accepted for publication

15 Jegier W Blankenship W & Lind J Venous pressure in the first hour of life and its relationship to placental transfusion *Acta Paediatr Scand 52* 485 1963

16 Judd S J Carter J N & Corcoran J M Circulating thyroid hormone concentrations and postural and venous compression *Br Med J IV* 735 1975

17 Kidd L Levison H Gemmel P Aharon A & Swyer P R Limb blood flow in normal and sick newborn *Am J Dis Child 112* 402 1966

18 Krasilnikoff P A *Albumin metabolism in normal mature and premature children* FADL Copenhagen 1975 Thesis

19 Lagerkranz H & Bistoletti P Catecholamine release in the newborn infant at birth *Pediatr Res 11* 889 1973

20 Larsen P R & Broskin K Thyroxine immunoassay using filter paper blood samples for screening of neonates for hypothyroidism *Pediatr Res 9* 604 1975

21 Parving H H Klebe J G & Ingomar C J Simultaneous determination of plasma volume and transcapillary escape rate with [131]I labeled albumin and T 1824 in the newborn *Acta Paediatr Scand 6?* 248 1973

22 Prince H P & Ramsden D B A new theoretical description of the binding of thyroid hormones by serum proteins *Clin Endocrinol 7* 307 1977

23 Redding R A & Pereira C Thyroid function in respiratory distress syndrome of the newborn *Pediatrics 54* 423 1974

24 Rose H Russel K P & Starr P Serum protein bound iodine of mothers and infants at delivery in premature and term pregnancies *Am J Obstet Gynecol 86* 767 1963

25 Siegel S *Non parametric statistics for the behavioral sciences* McGraw Hill Kogakusha Ltd Tokyo 1956

26 Tulzer H *Uber Beziehungen zwischen* Placenta und fetalen Serumeiweissbild *Arch Gynakol 19?* 7 1959

27 Usher R Shephard M & Lind J The blood volume of the newborn infant and placental transfusion *Acta Paediatr Scand 52* 497 1963

28 Walfish P G Screening for neonatal hypothyroidism using dried capillary blood method Observations on methodologic factors selection criteria and preliminary results *In* D A Fisher & G N Burrow (eds) *Perinatal thyroid physiology and disease* Raven Press Publ New York 1975 p 239

Submitted May 13 1978
Accepted July 11 1978

(B B J) The University Clinic of Paediatrics
Children s Hospital Fuglebakken
Drosselvej 57
2000 Copenhagen F
Denmark

Acta Pædiatr Scand 68 49–55 1979

SERUM CONCENTRATIONS OF THYROXINE BINDING GLOBULIN PREALBUMIN AND ALBUMIN IN HEALTHY FULLTERM SMALL FOR GESTATIONAL AGE AND PRETERM NEWBORN INFANTS

B BROCK JACOBSEN B PEITERSEN H J ANDERSEN and L HUMMER

From the University Clinic of Paediatrics Children s Hospital Fuglebakken and from the Department of Nuclear Medicine Rigshospitalet Copenhagen Denmark

ABSTRACT Jacobsen B B Peitersen B Andersen H J and Hummer L (The University Clinic of Paediatrics Children s Hospital Fuglebakken and the Department of Nuclear Medicine Rigshospitalet Copenhagen Denmark) Serum concentrations of thyroxine binding globulin prealbumin and albumin in healthy fullterm small for-gestational age and preterm newborn infants Acta Paediatr Scand 68 49 1979.—Simultaneous serum concentrations of thyroxine binding globulin (TBG) prealbumin (TBPA) and albumin (Alb) were measured in 130 fullterm 32 small for-gestational age and 25 preterm infants during their first six days of life In all infants serum concentrations of TBG were higher and serum TBPA and Alb were lower than in male adults Even higher serum TBG levels wer found in the mothers There was no correlation between serum concentrations in paired maternal and cord sera In infants with birth weights appropriate for gestation serum TBG TBPA and Alb concentrations increased progressively with gestational age In small for-gestational age infants born at term serum concentrations of TBG and Alb were lower than those in fullterm but higher than those in premature newborns Serum TBPA in small for-gestational age babies was evne lower than seen in prematures A positive correlation was found between thyroid hormones and TBG concentrations not between serum TBPA and thyroid hormones The ratios between serum concentration of thyroid hormones and proteins might indicate that more thyroid hormonebinding sites are occupied in fullterm than in low birth weight newborns However the main reason for the different serum levels of thyroid hormones in fullterm small for gestational age and preterm babies is probably the various serum TBG concentrations demonstrated in these infants

KEY WORDS Thyroxine binding globulin prealbumin albumin SGA infants preterm infants

Thyroid hormones in human serum are mainly bound to thyroxine binding globulin (TBG) prealbumin (TBPA) and albumin (Alb) (3 14 23) It has been shown that serum thyroxine concentration in fetuses (7) and in cord blood (20) increases with gestational age This relationship seems—at least in part—to be caused by variation in serum TBG binding capacity (1 7) However Oddie et al (20) did not find a correlation between gestational age and TBG concentration in cord sera from infants of 30 to 45 weeks gestation No data on any small for gestational age infants were given

Recently we reported that serum concentrations of thyroxine (T_4) and triiodothyronine (T_3) are lower in small for gestational age (SGA) babies born at term than in mature full term (FT) newborns even lower serum concentrations were seen i preterm (PT) infants with birth weights appropriate for gestation (12)

The present investigations were undertaken to measure the serum concentrations of TBG TBPA and Alb in healthy newborn infants of various maturity and gestational age and to study the relationship between the protein and thyroid hormone concentrations in these babies

Table 1 *Clinical data of fullterm (FT) small for gestational age (SGA) and preterm (PT infants aged 0 to 6 days*

Infants	No of infants (*n*)	Gestational age (weeks)		Birth weights (g)	
		Median	Range	Median	Range
FT	130	39	(37–42)	3 500	(2 800–4 500)
SGA	32	39	(37–43)	2 450	(1 520–2 900)
PT	25	34	(25–36)	2 185	(1 570–2 750)

MATERIAL AND METHODS

A total number of 187 healthy neonates of 25 to 43 weeks gestation were included in the study (Table 1) Gestational age and maturity were assessed as reported elsewhere (11 12) All patients with respiratory distress syndrome or asphyxia (Apgar score ≤6 after 1 min) were excluded Postnatal age ranged from 0 to 6 days All the PT infants had birth weights appropriate for gestation The birth weights differed significantly in the three groups of newborns ($p<0\ 001$) Low birth weight infants were usually placed in incubators All infants were fed with human milk

Usually blood was drawn from a peripheral vein Cord blood was taken from 36 babies at birth Only one blood sample was obtained from each infant The parents were informed and consent obtained Venous blood was also obtained from 20 mothers at the delivery Blood was centrifuged immediately and serum frozen at −20 until analyses were performed All analyses were done in duplicate The thyroid hormone and thyrotropin concentrations in sera from these infants and mothers have previously been reported by us (12)

The *thyroid hormone binding protein* concentrations were measured using Laurell s rocket immunoelectrophoresis (16) in 1% (w/v) antibody containing agarose gel 2–3 V/cm for 15 hours at 15 in Trisbarbitone buffer pH 8 6 Antibodies against TBG TBPA and Alb were obtained from Dakopatts Copenhagen Denmark When measuring serum TBPA and serum Alb the rockets were visualized by staining the gel with Coomassie Brilliant Blue The within assay coefficient of variation was 2% and the between assay coefficient of variation was 3% for both assays The antibody against TBG was not monospecific after preincubation of the sera with ^{125}I thyroxine (Amersham England 200 μCi/4 3 μg/ml) the precipitate of TBG was visualized by autoradiography (5 days) as reported by Nielsen et al (19) The within assay coefficient of variation for the TBG assay was 3% and the between assay coefficient of variation was 4%

The results were presented in terms of those obtained in a reference serum The reference serum was a serumpool from 100 apparently healthy male blood donors and the TBG TBPA and Alb concentrations in this serum were arbitrarily set to 100 units per liter Using routine laboratory methods the absolute concentrations of TBPA and Alb in the reference serum are 297 mg/l and 43 000 mg/l respectively The absolute TBG concentration measured[1] by radioimmunoassay is 9 3 mg/l

In the statistical analyses the Kruschal Wallis test th Mann Whitney test and Spearman rank correlation coefficient (R) were used (25)

RESULTS

Table 2 lists median value and interquartil range of serum TBG TBPA Alb and thyroi hormone concentrations in the differer groups of neonates The protein concentra tions were significantly higher in FT than i low birth weight infants Furthermore serui TBG and serum Alb levels were higher an serum TBPA lower in SGA compared wit those in PT newborns

The serum TBG concentrations in infan and mothers were much higher and seru TBPA and Alb lower than in the referenc serum (Table 2) The serum TBG in mothe was significantly higher than in infant ($p<0\ 01$) The serum concentrations of thy roid hormone binding proteins were stuc ied in 20 paired maternal and cord sera Th correlation coefficient R for serum TBG i mothers versus serum TBG in infants wa 0 11 the corresponding correlation coeff cients for serum TBPA and serum Alb wer 0 27 and 0 31 respectively Thus no signifi cant correlations were seen ($p>0\ 1$)

The serum levels of thyroid hormone binc ing proteins in the neonates (Fig 1) *did nc change significantly during the first week o life However some low birth weight infant* aged 5–6 days had higher serum values tha

[1] Kindly performed by J Kohrle D Sc Department c Endocrinology University of Hannover West Germany

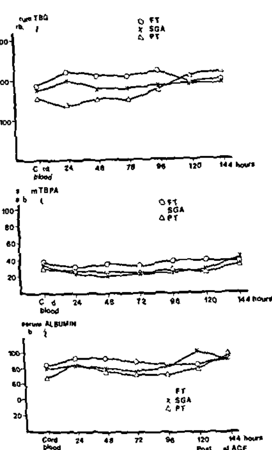


Fig 1 Variation of serum thyroxine binding globulin (TBG) prealbumin (TBPA) and albumin concentrations with postnatal age in fullterm (FT) small for gestational age (SGA) and preterm (PT) newborn infants Median values of protein concentration for each age group are shown No statistically significant postnatal changes were observed (Arb u /l is concentration in term of values in a reference serum (a serum pool from male adults which is stated to 100 arbitrary units per liter absolute concentrations—see text)

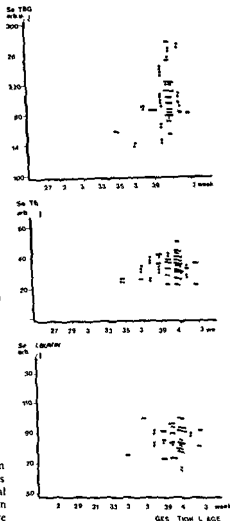


Fig 2 Variation of serum thyroxine binding globulin (TBG) prealbumin (TBPA) and albumin concentrations with gestational age in fullterm (FT) small for gestational age (SGA) and preterm (PT) newborn infants (Arb u /l —see legend to Fig 1)

the other infants during the first postnatal days Differences in birth weights might be one reason for this because serum protein concentrations seem to increase with birth weight The correlation coefficients (*R*) for TBG versus birth weight TBPA versus birth weight and Alb versus birth weight were 0 30 0 30 and 0 20 respectively ($p<0\ 01$) Al though statistically significant the correlations were obviously not very strong

Fig 2 shows serum TBG serum TBPA and serum Alb concentrations in infants with birth weights appropriate for gestation plotted ver sus gestational age in weeks The correlation coefficients (*R*) for TBG versus gestational age were 0 43 0 26 and 0 22 respectively ($p<0\ 003$) Thus the correlations of serum proteins with gestational age were of the same magnitude as with birth weights

In the group of SGA infants serum concen trations of thyroid hormone binding proteins did not show any correlation with birth weights or gestational age

Table 1 Clinical data of fullterm (FT) small for gestational age (SGA) and preterm (PT) infants aged 0 to 6 days

Infants	No of infants (n)	Gestational age (weeks)		Birth weights (g)	
		Median	Range	Median	Range
FT	130	39	(37–42)	3 500	(2 800–4 500)
SGA	32	39	(37–43)	2 450	(1 520–2 900)
PT	25	34	(25–36)	2 185	(1 570–2 750)

MATERIAL AND METHODS

A total number of 187 healthy neonates of 25 to 43 weeks gestation were included in the study (Table 1) Gestational age and maturity were assessed as reported elsewhere (11 12) All patients with respiratory distress syndrome or asphyxia (Apgar score ≤6 after 1 min) were excluded Postnatal age ranged from 0 to 6 days All the PT infants had birth weights appropriate for gestation The birth weights differed significantly in the three groups of newborns ($p<0\ 001$) Low birth weight infants were usually placed in incubators All infants were fed with human milk

Usually blood was drawn from a peripheral vein Cord blood was taken from 36 babies at birth Only one blood sample was obtained from each infant The parents were informed and consent obtained Venous blood was also obtained from 20 mothers at the delivery Blood was centrifuged immediately and serum frozen at −20° until analyses were performed All analyses were done in duplicate The thyroid hormone and thyrotropin concentrations in sera from these infants and mothers have previously been reported by us (12)

The *thyroid hormone binding protein* concentrations were measured using Laurell s rocket immunoelectrophoresis (16) in 1% (w/v) antibody containing agarose gel 2–3 V/cm for 15 hours at 15 in Trisbarbitone buffer pH 8 6 Antibodies against TBG TBPA and Alb were obtained from Dakopatts Copenhagen Denmark When measuring serum TBPA and serum Alb the rockets were visualized by staining the gel with Coomassie Brilliant Blue The within assay coefficient of variation was 2% and the between assay coefficient of variation was 3% for both assays The antibody against TBG was not monospecific after preincubation of the sera with ^{125}I thyroxine (Amersham England 200 μCi/4 3 μg/ml) the precipitate of TBG was visualized by autoradiography (5 days) as reported by Nielsen et al (19) The within assay coefficient of variation for the TBG assay was 3% and the between assay coefficient of variation was 4%

The results were presented in terms of those obtained in a reference serum The reference serum was a serumpool from 100 apparently healthy male blood donors and the TBG TBPA and Alb concentrations in this serum were arbitrarily set to 100 units per liter Using routine laboratory methods the absolute concentrations of TBPA and Alb in the reference serum are 297 mg/l and 43 000 mg/l respectively The absolute TBG concentration measured by radioimmunoassay is 9 3 mg/l

In the statistical analyses the Kruschal Wallis test the Mann Whitney test and Spearman rank correlation coefficient (*R*) were used (25)

RESULTS

Table 2 lists median value and interquartile range of serum TBG TBPA Alb and thyroid hormone concentrations in the different groups of neonates The protein concentrations were significantly higher in FT than in low birth weight infants Furthermore serum TBG and serum Alb levels were higher and serum TBPA lower in SGA compared with those in PT newborns

The serum TBG concentrations in infants and mothers were much higher and serum TBPA and Alb lower than in the reference serum (Table 2) The serum TBG in mothers was significantly higher than in infants ($p<0\ 01$) The serum concentrations of thyroid hormone binding proteins were studied in 20 paired maternal and cord sera The correlation coefficient *R* for serum TBG in mothers versus serum TBG in infants was 0 11 the corresponding correlation coefficients for serum TBPA and serum Alb were 0 27 and 0 31 respectively Thus no significant correlations were seen ($p>0\ 1$)

The serum levels of thyroid hormone binding proteins in the neonates (Fig 1) *did not change significantly during the first week of life* However some low birth weight infants aged 5–6 days had higher serum values than

---

Kindly performed by J Kohrle D Sc Department of Endocrinology University of Hannover West Germany

um T_3 (nmol/l)	
dian ce	Inter quartile range
	(1 57–3 11)
)	(1 35–2 33)
3	(0 68–1 45)
0 01	
1	(1 95–? 75)

ratios were significantly different in the three groups of newborn infants the highest values appeared in FT and the lowest values were seen in PT babies Similar results were obtained concerning T_4/Alb and T_3/Alb ratios The ratios between serum T_4 and TBG concentrations were significantly higher in infants than for maternal values ($p<0\ 01$) This was less evident for ratios between serum T_3 and TBG values

DISCUSSION

The present study demonstrates that in newborn infants serum TBG concentration is higher and serum TBPA and serum Alb are lower than in male adults (i e in our reference serum) Furthermore our data confirm the findings that even higher serum TBG concentrations appear in the mothers at delivery (4 5 18 24) However there was no correlation between serum concentrations of thyroid hormone binding proteins in paired cord and maternal sera indicating very little placental transfer of these proteins

It has been shown that in human fetuses of 8 to 24 weeks gestation the serum binding capacity of TBG increases with gestational age corresponding to a relative decrease in binding capacity of TBPA and Alb (1 7) Nevertheless in some earlier studies the serum binding capacity of TBG in premature babies has been found to be the same as in mature infants (17 22) In a recent study by Oddie and associates the cord blood TBG concentrations did not vary significantly with gestational age (20) In that study serum TBG was measured by radioimmunoassay the gestational age of the infants ranged from 30 to 45 weeks and it was suggested that most of the increase in TBG in fetal serum occurs prior to 30 weeks gestation (20) We found in newborns with *birth weights appropriate for gestation* that serum concentrations of TBG TBPA and Alb increased progressively with gestational age In the group of infants who were *small for gestational age* the serum concentration of thyroid hormone binding proteins did not show any correlation with birth weight or gestational age probably because that group was very heterogeneous concerning birth weights in relation to gestational age One reason for the differences in the results of previous investigations (17 20 22) and the present study could be that we made a distinction between FT and SGA newborns Different methodology and various gestational age of the infants might also influence the results In a study of serum concentrations of total protein and albumin Bergstrand et al (2) found lower serum concentrations in SGA than in FT but higher values than in PT neonates findings which are in accordance with our data The present finding of a lower serum TBPA level in SGA than in other groups of newborns has not been recorded previously and needs further investigations

The causes of different serum levels of thyroid hormone binding proteins in infants with various gestational age and maturity are unknown The increase in serum levels in infants with birth weights appropriate for gestation might be due to a progressive increase in protein synthesis with gestation This does not explain the lower serum protein levels in SGA compared to FT neonates These are comparable to finding of decreased thyroid hormone

Table 2 *Serum concentrations of thyroxine binding globulin (TBG) prealbumin (TBPA) albumin (Alb) and thyroid hormones in newborn babies aged 0–6 days and in mothers at delivery*
Abbreviations—see Table 1

Infants	Serum TBG (arb u /l) Median value	Inter quartile range	Serum TBPA (arb u /l) Median value	Inter quartile range*	Serum Alb (arb u /l) Median value	Inter quartile range*	Serum T_4 (nmol/l) Median value	Inter quartile range*
FT (n=130)	200	(182–227)	36	(30–40)	88	(81–93)	236	(180–[illegible]78)
SGA (n=32)	175	(150–204)	23	(21–32)	81	(76–89)	193	(1[illegible]8–[illegible]50)
PT (n=25)	154	(139–176)	26	(22–29)	77	(65–90)	145	(119–184)
Kruschal Wallis test	$p<0\ 001$		$p<0\ 001$		$p<0\ 001$		$p<0\ 001$	
Mothers (n=20)	277	(260–301)	71	(59–88)	80	(70–84)	175	(1[illegible]8–[illegible]4[illegible]

* Interquartile range signifies the range between the quartile of the lowest values and the quartile of the highest (i e fifty per cent of the distribution)

In infants with birth weights appropriate for gestation (i e FT and PT) serum TBG was correlated with serum Alb (R 0 39 $p<0\ 001$), but not correlated with serum TBPA (R 0 04) Comparable observations were made in SGA newborns

The thyroid hormone binding protein levels in sera from FT SGA and PT neonates differed in the same way as the thyroid hormone levels (Table 2) and investigations of the relation between these variables were therefore performed Only infants two to six days of age were included in the calculations because pronounced changes in total and free thyroid hormone concentrations appear during the first 24 hours after birth (6 12) A good correlation was found in all groups of infants between thyroid hormone and TBG concentration The correlation coefficients for serum T_4 ve sus serum TBG were 0 68, 0 57, and 0 60 f FT SGA and PT infants ($p<0\ 001$) The cc responding values for serum T_3 versus seru TBG were 0 35 0 54 and 0 58 respective ($p<0\ 02$) There was no correlation betwe thyroid hormone and TBPA concentrations any groups In FT and SGA infants but not PT babies the thyroid hormone seemed to l related to Alb concentrations but the correl tions were less strong (R 0 36 to 0 45)

In an attempt to evaluate the saturation hormone binding sites the individual serum and serum T_3 were divided with correspondin serum TBG concentrations median values a shown in Table 3 Both T_4/TBG and T_3/TB

Table 3 *Median values of ratios between serum concentrations of thyroid hormones an thyroxine binding globulin and albumin in infants 2 to 6 days of age and in mothers at deliver*
Abbreviations—se Table 1

Ratio×10^2	Newborn babies FT (n=92)	SGA (n=26)	PT (n=19)	Kruschal Wallis test	Mothers (n=20)
T_4/TBG	118	112	95	$p<0\ 05$	64
T_4/Alb	270	237	225	$p<0\ 05$	233
T_3/TBG	1 09	1 07	0 69	$p<0\ 001$	0 79
T_3/Alb	2 67	2 47	1 65	$p<0\ 001$	2 90

small for gestational age infants *Acta Pædiatr Scand 61* 1 8 197?
3 Bernal J & Refetoff S The action of thyroid hormone *Clin Endocrinol 6* 2 7 1977
4 De Nayer P Malvaux P Van Den Schrieck H G Beckers C & De Visscher M Free thyroxine in maternal and cord blood *J Clin Endocrinol 6* 233 1966
5 Dussault J Row V V & Lickrish G Studies of serum triiodothyronine concentration in maternal and cord blood Transfer of triiodothyronine across the human placenta *J Clin Endocrinol 29* 595 1969
6 Erenberg A Phelps D L Lam R & Fisher D A Total and free thyroid hormone concentrations in the neonatal period *Pediatrics 5?* ?11 1974
7 Greenberg A H Czernishow P Reba R C Tyson J & Blizzard R M Observations on the maturation of thyroid function in early fetal life *J Cl n Invest 49* 1790 1970
8 Hesch R D Gatz J Juppner H & Stubbe P TBG-dependency of age related variations of thyroxine and triiodothyronine *Horm Metab Res 9* 141 1977
9 Ingelbleek Y De Visscher M & De Nayer P Measurement of prealbumin as index of protein calorie malnutrition *Lancet II* 106 197?
10 Ingelbleek Y De Nayer P & De Visscher M Thyroxine binding globulin in infant protein-calorie malnutrition *J Clin Endocrinol Metab 39* 178 1974
11 Jacobsen B B Andersen H J Dige Petersen H & Hummer L Pituitary thyroid responsiveness to thyrotropin releasing hormone in preterm and small for gestational age newborns *Acta Paediatr Scand 66* 541 1977
1? Jacobsen B B Andersen H J Peitersen B Dige Petersen H & Hummer L Serum levels of thyrotropin thyroxine and triiodothyronine in fullterm small for gestational age and preterm newborn babies *Acta Pædiatr Scand 66* 681 1977
13 Koivisto M Simila S Haapalahti J & Reinila M Thyroid function assay using the effective thyroxine ratio in infancy *Scand J Cl n Lab Invest 33* 105 1974
14 Korcek L & Tabachnick M Thyroxine protein interactions *J B ol Chem 251* 3558 1976
15 Krasilnikoff P A *Albumin metabolism in normal mature and p ematu e children* FADL Copenhagen 1975
16 Laurell C B Quantitative estimations of proteins by electrophoresis in agarose gels containing antibodies *Anal Biochem 15* 45 1966
17 Lemarchand Beraud T Genazzani A R Bagnoli F & Casoli M Thyroid function in the premature and the fullterm newborn *Acta Endocrinol (Kbh) 70* 445 197?
18 Montalvo J M Wahner H W Mayberry W E & Lum R K Serum triiodothyronine total thyroxine and thyroxine to triiodothyronine ratios in paired maternal cord sera and at one week and one month of age *Pediatr Res 7* 706 1973
19 Nielsen H G Buus O & Weeke B A rapid determination of thyroxine binding globulin in human serum by means of the Laurell rocket immunoelectrophoresis *Clin Chim Acta 36* 133 1972
?0 Oddie T H Fisher D A Bernard B & Lam R W Thyroid function at birth in infants of 30 to 45 weeks gestation *J Pediatr 90* 803 1977
21 Pedersen K O A systematic study of variables affecting protein binding of thyroxine and triiodothyronine in serum *Scand J Clin Lab Invest 34* 47 1974
?2 Perry R E Hodgman J E & Starr P Maternal cord and serial venous blood Protein bound iodine thyroid binding globulin thyroid binding albumin and prealbumin in premature infants *Pediatrics 35* 759 1965
?3 Robbins J Structure and function of thyroxine transport proteins *In* I R Dysdale R Ramsdan & H Hoffemberg (eds) *Thyroid hormone metabolism* Academic Press London 1975
24 Robin N I Refetoff S Fang V & Selenkow H A Parameters of thyroid function in maternal and cord serum at term pregnancy *J Clin Endocrinol Metab* 29 1?79 1969
?5 Siegel S Nonparametric statistics for the behavioral sciences McGraw Hill Kogakusha Tokyo 1956

Submitted Febr 23 1978
Accepted May 3 1978

(B B J) The University Clinic of Paediatrics
Children s Hospital Fuglebakken
Drosselvej 57
?000 Copenhagen F
Denmark

binding levels in sera from protein calorie mal nutrition (9 10) It remains to clarify whether a lower protein synthesis or higher catabolism occur in SGA in comparison with other new born infants Other explanations may also be considered Krasilnikoff (15) has reported that the lower serum Alb concentration in PT babies might be explained by dilution of the total intravascular mass of albumin in prema tures because the total intravascular mass ex pressed per kg body weight were equal in the PT and FT newborns However the different serum protein levels in the present study can not exclusively be caused by dilution which would change the protein concentrations equ ally

The TBG is physiologically the most im portant carrier of thyroid hormones in blood with an affinity for T_4 and T_3 which is much greater than the affinity of TBPA and Alb for these hormones (3 14 21 23) This agrees with our finding of a very good correlation be tween serum concentrations of thyroid hor mones and TBG in the newborns Serum con centration of TBPA was very low and not cor related with serum TBG for which reason it was not surprising that no correlation was found between TBPA and thyroid hormone concentrations

The increased serum TBG concentration found in all newborn infants is probably the reason why the postnatal rise in free serum T_4 and T_3 concentrations is transitory (6) in spite of a more sustained increase in total thyroid hormone concentrations which appears in neonates (6 12 17) The thyroid hormone binding proteins are serving as reservoirs of hormones Previous in vitro studies have in dicated that the thyroxine binding proteins are slightly more saturated in newborns than in mothers (13 24) We confirmed the observa tions that the ratio between serum T_4 and TBG concentration is higher in infants than in adults (8) Furthermore as shown in Table 3 the ratios between serum T_4 as well as serum T_3 and TBG or Alb concentrations were signifi cantly different in the three groups of new borns Although the number of infants aged ? to 6 days in each group was rather small our data might indicate that more binding sites are occupied per unit protein in FT than in SGA babies even fewer binding sites are occupied in PT neonates Differences in body tempera ture and intravascular pH might possibly influ ence the hormone bindings (21) and one assumption is therefore that the free hormone concentrations and the affinity of the proteins for thyroid hormones are the same in the three groups of newborns In fact Greenberg et al (8) reported that T_4 binding characteristics of fetal TBG and free thyroid hormone concen trations in fetuses of 24 weeks gestations were like those in newborns

We have previously reported that the pitui tary TSH responses to exogenous TRH as well as the effect of endogenous TSH meas ured by thyroid hormone increases was of the same magnitude in low birth weight infants as in fullterm (11) The serum concentrations of TBG (and Alb) differed in FT SGA and PT neonates in the same way as the thyroid hor mone levels and are probably the main reasons for the various thyroid hormone levels in these infants Further investigations of the postnatal changes in hormone and serum protein con centrations are in progress

ACKNOWLEDGEMENTS

We wish to thank J Nyboe cand act Rigshospitalet for great help with the computer analyses Thanks to the staff members of the two laboratories involved particularly to Mrs K Jorgensen and Mrs A Wilsom and to the nurses of the Radiological Department Children s Hospital Fuglebakken The secretarial assistance of Miss A Jacob sen is greatly appreciated

The study was supported by grants from the Danish Medical Research Council and the Dagmar Marshall Foundation

REFERENCES

1 Andreoli M & Robbins J Serum proteins and thyroxine protein interaction in early human fetuses J Clin Invest 41 1070 1962
2 Bergstrand C G Karlsson B W Lindberg T & Ekelund H Alfafoetoprotein albumin and total pro tein in serum from preterm and term infants and

PRESSURE PASSIVE CEREBRAL BLOOD FLOW AND BREAKDOWN OF THE BLOOD–BRAIN BARRIER IN EXPERIMENTAL FETAL ASPHYXIA

H C LOU N A LASSEN W A TWEED G JOHNSON
M JONES and R J PALAHNIUK

From the Department of Anaesthesia Health Sciences Centre Winnipeg Canada

ABSTRACT Lou H C Lassen N A Tweed W A Johnson G Jones M and Palahniuk R. J (Department of Anaesthesia Health Sciences Centre Winnipeg Canada) Pressure passive cerebral blood flow and breakdown of the blood–brain barrier in experimental fetal asphyxia Acta Paediatr Scand 68 57 1979.—Cerebral blood flow (CBF) was studied in non-exteriorized near term sheep fetuses using the radioactive microsphere technique By partially occluding the umbilical vessels for a period of 1–1½ hours a progressive and severe asphyxia with a final arterial pH of 6 90 was achieved Varying the mean arterial blood pressure in the fetuses by blood withdrawal or infusion in this state CBF was measured at different perfusion pressures (mean arterial blood pressure (MABP) minus central venous pressure (CVP)) A passive flow/pressure relationship—loss of autoregulation—was found with hyperemia reaching CBF values up to 6 times normal at normal MABP of about 60 to 70 mmHg and severe ischemia reaching CBF values close to zero in large cortical areas at MABP of 30 mmHg CVP remained essentially unchanged at 10–15 mmHg The severe and prolonged asphyxia rendered the blood–brain barrier leaky to the albumin tracer Evans blue In four other fetuses umbilical cord clamping was omitted However only in one of these cases was acidosis completely avoided and CBF autoregulation maintained The three other fetuses were acidotic at the end of the surgical procedure and had impaired *autoregulation*

KEY WORDS Asphyxia autoregulation blood brain barrier cerebral ischemia fetal circulation hypotension hypoxia regional cerebral blood flow

Courville (5) has pointed to *cerebral ischemia* as a possible complicating factor contributing to brain damage in the distressed newborn Not only is the arterial blood poorly oxygenated but perhaps even the volume of this blood supply may be severely reduced The concept has gained support from the findings of Schwartz (28) that nearly all infants dying during the first days after birth have brain tissue infarcts located to border zones between arterial territories Direct evidence of brain ischemia in prolonged and severe asphyxia was obtained by Reivich et al (27) studying fetal monkeys

The immediate response to fetal asphyxia is *hypertension* But as shown by Dawes et al (6) *hypotension* eventually develops This we believe may be the mechanism leading to cerebral ischemia in asphyxia if normal autoregulation of cerebral blood flow is lacking The aim of the present study therefore has been to establish if cerebral blood flow is constant or not in the face of variations of arterial blood pressure in asphyxiated sheep fetuses

MATERIALS AND METHODS

The study involved 17 near term pregnant sheep The sheep were anaesthetized by intrathecal lumbal injection of 2–4 ml 2% lidocaine The abdomen was opened by a transverse incision in the right iliac region One upper and one lower extremity were successively delivered through small incisions in the uterus Care was taken not to interfere with placental circulation and to minimize the exposure of uterus and fetal limbs to the exterior environment A polyethylene catheter was introduced into a brachial artery the tip being placed in the brachio-cephalic trunk supplying upper extremities and the head Catheters were

Table 2 *CBF measurements in severely asphyxiated fetuses (see Figs 1 and 2 for a description of the main findings*

	pH	Pa_{CO_2} (mmHg)	Pa_{O_2} (mmHg)	MABP (mmHg)	CVP (mmHg)	rCBF (ml/g/min)	pH	Pa_{CO_2} (mmHg)	Pa_{O_2} (mmHg)	MABP (mmHg)	CVP (mmHg)	rCBF (ml/g/min)
Group A Decrease in arterial blood pressure												
II	6 89	58	1?	60	10	co 5 96 pv 14 2 bs 13 8 ce 6 60	6 83	65	15	50	10	co 3 84 pv 4 11 bs 3 84 ce 0.59
JJ	6 9	50	?	80	10	co 4 67 pv 10 4 bs 14 2 ce 8 18	6 9?	54	2_	40	10	co 1 10 pv 3 27 bs 4 ?9 ce 2 52
KK	6 78	80	15	55	10	co ? 11 pv 4 09 bs 4 03 ce ? 87	6 79	80	15	30	10	co 0 05 pv 0 08 bs 0 08 ce 0 03
LL	6 99	68	18	75	10	co 3 58 pv 8 56 bs 8 39 ce 4 67	6 99	71	18	55	10	co 1 32 pv 3 1? bs 4 38 ce 2 70
Mean	6 90	64	17	68	10	co 4 08 pv 9 31 bs 10 1 ce 5 58	6 88	68	17	44	10	co 1 58 pv 3 15 bs 3 15 ce 1 48
Group B Increase in arterial blood pressure												
BB	6 94	70	16	45	15	co 1 79 pv – bs 3 50 ce 2 8?	7 1?	39	31	85	15	co 0 864 pv – bs 1 68 ce 2 09
DD	6 83	59	1?	45	10	co 0 95 pv 2 48 bs 3 13 ce 1 71	6 75	48	28	75	10	co 1 75 pv 3 8? bs 4 98 ce 2 00
FF	6 84	54	13	50	15	co 1 98 pv 4 00 bs 4 70 ce 2 48	6 85	54	24	90	15	co 2 10 pv 4 31 bs 4 83 ce 2 24
HH	6 9	4?	8	50	10	co 1 40 pv 4 20 bs 4 ?6 ce 2 7_	6 9?	4?	?8	60	10	co 1 40 pv 2 79 bs 3 0 ce 3 78
Mean	6 88	56	17	47	13	co 1.54 pv 3 56 bs 3 90 ce ? 83	6 84	48	?7	75	13	co 1 75 pv 3 64 bs 4 33 ce 2 51

BB excluded as cuff was released co Cortex pv Periventricular region bs Brain stem ce Cerebellum MABP Mean aretnal blood pressure CVP Central venous pressure rCBF Regional cerebral blood flow

ments (JJJ and MMM) Approximately 10^6 spheres with 4×10^6 counts were injected during continuous agitation from a special vial into the inferior vena cava The concentration of microspheres in the blood on its way to the brain was measured by counting 5 ml blood withdrawn continuously during a period of 2 min from the brachiocephalic trunk The blood sample was counted in a Chicago® well type nuclear gamma spectrometer The injection of the second batch of microspheres (the order randomly chosen) took place approximately 10 min later

rCBF was calculated from the formula

$$CBF = \frac{F \quad tissue}{i}$$

(F Sampling rate of arterial blood i_{tissue} and i counting rates of cerebral tissue and of arterial blood)

Table 1 *Control state after termination of surgical procedure*

Sheep	Weight (kg)	pH	Pa_{CO_2} (mmHg)	Pa_{O_2} (mmHg)	MABP (mmHg)	CVP (mmHg)
BB	3 1	7 35	35	19	55	12
DD	4 5	7 24	34	16	75	15
FF	4 1	7 35	35	17	80	15
HH	4 0	7 38	27	21	50	10
II	4 2	7 31	40	14	75	10
JJ	4 6	7 36	36	14	75	10
KK	4 2	7 22	44	14	65	10
LL	3 7	7 31	34	22	60	10
JJJ	3 0	7 27	36	18	58	10
LLL	3 0	7 19	38	17	76	10
MMM	30	7 33	30	19	55	10
PPP	2 8	7 25	24	24	60	10
Mean	3 7	7 30	34	18	65	11
S D	0 66	0 06	5 4	3 3	10	2 0
S E	0 19	0 02	1 6	0 9	3 0	0 6

In these acute preparations fetal hypocapnia was a general finding probably due to maternal hyperventilation Several fetuses were hypoxic and/or slightly acidotic

also placed in the inferior vena cava (from femoral vein) and in a femoral artery Both were connected to pressure transducers (Statham*) and in this way mean arterial blood pressure (MABP) and central venous pressure (CVP) were recorded continuously pH was monitored at least once every 10 min and P_{O_2} and P_{CO_2} using Radiometer* electrodes at more irregular intervals in arterial blood samples from the brachio-cephalic trunk The values were corrected for temperature Rectal temperature was measured in the ewes All were normothermic After positioning the catheters a small inflatable cuff was placed around the umbilical cord in eight fetuses The surgical procedure was then terminated by suturing the abdomen

State after termination of the surgical procedure

After completion of the surgical procedure the state of the 12 fetuses was determined (Table 1) These acute preparations were all hypocapnic and several slightly acidotic and hypoxic (Normal values in chronic preparations pH 7 345±0 006 Pa_{O_2} 21 4±1 0 Pa_{CO_2} 53 8±0 9 (23)) probably due to maternal hyperventilation and stress during the operation These deviations were considered to be of minor importance in view of the fact that a similar state is typical in relation to normal birth

Induction of severe and prolonged asphyxia

In eight experiments the non-exteriorized fetus was gradually asphyxiated by inflating the umbilical cuff to a pressure near the mean arterial blood pressure The fetuses reacted initially by hypertension with a mean increase of 19 mmHg The maximal MABP was reached after 30 min and in most cases maintained during another 30 min Then the MABP gradually fell When pH had reached values of about 6 90 the MABP had returned to the initial levels of 60–75 mmHg In four of these cases (group A Table 2) the first CBF measurement took place at this point 60–90 min after starting partial cord constriction The measurement was repeated about 10 min later after the MABP had been lowered to a mean of 44 mmHg by withdrawal of 40–50 ml blood

In 4 other fetuses which were asphyxiated for a similar period of time and to a similar degree (group B Table 2) the blood withdrawal was carried out immediately before the first CBF measurement which took place at a MABP of 47 mmHg (mean value) The second measurement was carried out about 10 min later after re infusion of blood to a mean MABP of 75 mmHg

Fetuses without cord constriction

In the remaining 4 fetuses (Table 3) no attempt was made to induce asphyxia After the surgical procedure had been terminated one fetus had normal arterial pH (MMM) the other three were slightly acidotic (Table 1) At the time of the first CBF measurement (a few minutes later) all except MMM were moderately hypoxic CBF was measured at different MABPs as described above However it proved to be considerably more difficult to achieve significant blood pressure changes by bleeding or re infusion in these less asphyxiated fetuses 90–110 ml of blood had to be withdrawn or infused to obtain appreciable pressure changes

In two experiments three types of isotope labelled microspheres were used allowing three CBF measurements at different MABPs (see below)

Determination of rCBF

The inert microsphere technique as described by Hales (14) was used This newly developed method is now regarded as the most accurate method in experimental animals (16) The microspheres were made of plastic material having a specific gravity slightly greater than that of whole blood (1 1–1 6 compared with approximately 1 05) with a diameter of 15 μm In most cases two batches were used one labelled with ^{85}Sr one with ^{141}Ce (3M Company St Paul) allowing two CBF measurements in each experiment In two experiments also ^{51}Cr marked microspheres were used allowing three CBF measure

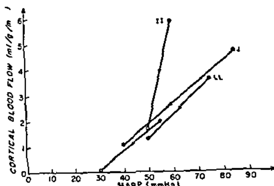


Fig 1 Cortical blood flow (average of 8 determinations) measured at normal MABP and subsequently at decreased MABP in each of 4 sheep fetuses Very high flows are recorded in severe normotensive asphyxia but CBF drops proportionally or even more as MABP decreases Autoregulation is abolished (Table 2 A)

very high up to 5 9 ml/g/min in cortex and 14 ml/g/min in the periventricular regions and brain stem These values are 4–6 times normal (23) and indicate an extreme decrease in vascular resistance Vasodilatation After induction of moderate or severe hypotension the flows fall dramatically CBF is pressure passive In other words normal autoregulation is abolished

Table 2B illustrates the CBF in severe hypotensive asphyxia followed by induction of normotension after re infusion of blood It is seen that restoration of normotension fails to raise CBF appreciably in these severely asphyxiated fetuses This phenomenon may be termed false autoregulation and is also seen in other pathological states of the brain (3) It is probably due to increased oedema formation and raised intracranial pressure

Table 3 shows the CBF at different MABPs in the fetuses without cord constriction In the case of the best preserved fetus (MMM) flow is essentially unchanged at decreased and increased MABP Autoregulation is preserved PPP which was still acidotic had completely lost autoregulation The 2 other fetuses were less affected

The comparative distribution of CBF in different regions of the brain (periventricular region cerebellum and brain stem) show a consistent pattern The regions near the neuraxis (periventricular region and brain stem) have by far the highest flows About 2 5 times that of the cortex (Tables 1 2 and 3)

In vivo injection of Evans blue at the termination of prolonged and severe asphyxia resulted in a marked coloration throughout the basal ganglia and especially the cortex indicating a breakdown of the blood-brain barrier to albumin

In the control twins Evans blue was visible only in major veins

DISCUSSION

The present demonstration of extremely high CBF in *normotensive* severe fetal asphyxia is expected As mentioned in the introduction *increased* CBF is the usual consequence of asphyxia as both hypoxia and hypercapnia with increased extracellular acidosis are important vasodilator stimuli (9 18 19) Even CBF prior to cord constriction is in accordance with Makowski et al much higher than in normal adults probably due to the fact that fetal blood is much less oxygenated (23)

If autoregulation is preserved hypotension will not produce any change in CBF or in the concentrations of energy rich phosphates in the brain provided the hypotension does not become excessive (1 2 18 20 26) We found that a moderate decrease in the mean arterial blood pressure leads to a proportional (or even more pronounced) decrease in the very high cerebral blood flow of the severely asphyxiated fetus This must constitute a breakdown of a compensatory mechanism leading to an increase in tissue hypoxia and acidosis

The failure of seeing a flow increase when the reversing order of the experiment (group B Table 2) is surprising Since impaired autoregulation was seen even in minimally asphyxiated control studies (Table 3 Fig 3) the constancy of flow in group B might be related to the CBF constancy-despite variation in perfusion pressure seen in the most severely dam

Table 3 *Fetuses without cord constriction (see Fig 3 for a description of the main findings)*

	pH	Pa_{CO_2} (mmHg)	Pa_{O_2} (mmHg)	MABP (mmHg)	CVP (mmHg)	rCBF (ml/g/min)
First CBF measurement						
JJJ	7 33	32	14	54	10	co 1 66 pv 2 06 bs 2 44 ce 2 21
LLL	7 34	28	15	75	10	co 1 69 pv 2 50 bs 3 46 ce 2 93
MMM	7 33	30	19	55	10	co 0 98 pv 1 29 bs 1 66 ce 1 63
PPP	7 28	38	17	60	10	co 1 45 pv 2 57 bs 3 00 ce 2 22
Second CBF measurement						
JJJ	7 35	30	17	45	10	co 1 11 pv 1 42 bs 1 32 ce 1 52
LLL	7 32	30	17	60	10	co 0 96 pv 1 49 bs 2 28 ce 1 97
MMM	7 34	28	22	45	10	co 1 08 pv 1 42 bs 1 90 ce 1 88
PPP	7 27	39	21	48	10	co 0 56 pv 0 89 bs 1 16 ce 1 01
Third CBF measurement						
JJJ	7 31	29	17	55	10	co 1 67 pv 2 44 bs 2 33 ce 2 44
MMM	7 33	28	23	64	10	co 1 21 pv 2 24 bs 2 56 ce 2 36

Blood–brain barrier studies

After the last rCBF measurement 12 ml of a 2% aqueous solution of the albumin tracer Evans blue was injected slowly intravenously (2 min injection time) The tracer was allowed to circulate for 15 min In two cases the presence of a twin fetus made it possible to perform a control study on the non asphyxiated fetus

After termination of the experiment the ewe and the fetus were killed by intravenous injection of barbiturate followed by KCl The fetal brain was removed and 1–2 g samples were taken for counting from the following regions 4 samples from the left hemisphere cortex 4 from the right hemisphere cortex 1 from the periventricular area (thalamus hypothalamus basal ganglia and adjacent white matter) 1 from the brain stem and 1 from cerebellum The tubes of the spectrometer were filled to a height of less than 2 cm and counted in the same manner as the blood samples (14) At autopsy the correct placement of catheters was ascertained

RESULTS

Fig 1 and Table 2 A show the regional CBF in severe normotensive asphyxia The flows are

mature infants with low serum bilirubin concentrations *Pediatrics 45* 906 1970
13 Haggendal E & Johansson B On the pathophysiology of increased cerebrovascular permeability in acute arterial hypertension in cats *Acta Neurol Scand 48* 65 1972
14 Hales J R S Radioactive microsphere techniques for studies of the circulation *Clin Exp Pharmacol Physiol* Suppl *1* 31 1974
15 Hambleton G & Wigglesworth J S Origin of intraventricular haemorrhage in the preterm infant *Arch Dis Child 59* 651 1976
16 Heistad D O Marcus M L & Mueller S Measurement of cerebral blood flow with microspheres *Arch Neurol 34* 657 1977
17 Johansson B Blood–brain barrier dysfunction in acute arterial hypertension after papaverine induced vasodilatation *Acta Neurol Scand 50* 573 1974
18 Johansson H & Siesjo B K Cerebral blood flow and oxygen consumption in the rat in hypoxic hypoxia *Acta Physiol Scand 93* ?69 1975
19 Lassen N A Brain extracellular pH The main factor controlling cerebral blood flow *Scand J Clin Lab Invest 22* ?47 1968
0 Lassen N A Discussion In *Cerebral Blood Flow Regulation* Proc Fifth Intern Symp Roma–Siena 1971 *European Neurol 8* 2?9 1972
21 Leech R W & Alvord E C Morphological variations in periventricular leukomalacia *Am J Pathol 74* 591 1974
? Lou H C Tweed W A Johnson G Jones M & Lassen N A Break-down of the blood–brain barrier in kernicterus *Lancet* i 106? 1977
23 Makowski E L Schneider J M Tsoulos N G Colwill J R Battaglia F C & Meschia G Cerebral blood flow oxygen consumption and glucose utilization of fetal lamils in utero *Am J Obstet Gyne col 114* ?9? 1972
?4 Mossakowski M J Long D M Myers R E Rodriquez de Curet H & Klatzo I Early histochemical changes in perinatal asphyxia *J Neuropath Exp Neurol 27* 500 1968
25 Myers R E Four patterns of perinatal brain damage and their conditions of occurrence in primates In B S Meldrum & D C Masden (eds) *Advances in Neurology* Raven Press *10* 2 3 1975
6 Purves J M & James I M Observations on the control of cerebral blood flow in the sheep fetus and newborn lamb *Circ Res 25* 651 1969
?7 Reivich M Brann A W Shapiro H M & Myers R E Regional cerebral blood flow during prolonged partial asphyxia *In* J S Meyer (ed) *Research on Cerebral Blood Flow* Ch C Thomas Springfield Ill 197? p ?17
?8 Schwartz P Birth injuries *In* J Minkler *Pathology of the Nervous System* McGraw Hill N Y 1971 p 1779

Submitted Jan 7 1978
Accepted June 0 1978

(H C L) Department of Paediatrics GN 50 4
Rigshospitalet Blegdamsvej 9
DK ?100 Copenhagen
Denmark

aged cases of stroke and brain trauma (10 11) (false autoregulation) and it is speculated that the *local tissue pressure* rises during arterial pressure increase to offset an increase in perfusion pressure

The finding that even minimal acidosis impairs autoregulation in the fetus is also unexpected The explanation may well be that the arterioles already are somewhat dilated in the normal fetal state due to arterial hypoxia in comparison to the postnatal state

Our experimental demonstration of critically low cerebral perfusion in hypotensive perinatal distress would explain why hypoxic tissue damage is mainly seen in watershed regions between vascular territories in the brain (7 8, 21 25)

The fact that autoregulation is lacking in the hypoxic fetus will render the capillary walls more directly exposed to an eventual increase in arterial blood pressure and in the germinal layer of the very immature brain the rich capillary network is not supported by rigid glial structures (15) This may well explain why germinal layer capillary bleeding is a frequent complication to cerebral hypoxia in the premature infant

Blood–brain barrier damage

The present study also demonstrated that prolonged severe asphyxia leads to pronounced damage to the blood–brain barrier in particular in the cortex and to a lesser extent in the basal ganglia Mossakowski et al have previously reported rare foci of blue coloration following Evans blue injection after short lasting total asphyxia in monkeys (24) The dye was seen primarily inside the neurones

It is most likely that the breakdown of the blood—brain barrier is not caused directly by the tissue hypoxia but to its indirect effect Maximal vasodilatation in combination with moderate arterial hypertension The breakdown of the blood–brain barrier and oedema may further aggravate circulatory disturbances in severe and prolonged asphyxia (13 17)

The passage of albumin from plasma to neurones will enhance transport of albumin bound bilirubin to the neurones and this offers an explanation of the increased susceptibility of small asphyxiated infants to kernicterus (12 22)

ACKNOWLEDGEMENTS

The main part of the experimental work took place during H C Lou s tenure as visiting professor of pediatric neurology at the *University of Manitoba* The generous support of that institution as well as of the Dagmar Marshall Foundation is acknowledged We gratefully acknowledge the financial support of the Province of Manitoba Department of Health and Social Development and the Seller s *Foundation of Manitoba*

REFERENCES

1 Brierley J B Brown A W Excell B J & Meldrum B S Brain damage in the rhesus monkey resulting from profound arterial hypotension *Brain Res 13* 68 1969

2 Brierley J B & Excell B J The effects of profound systemic hypotension upon the brain of Macacus rhesus *J Physiol* (Lond) *175* 50P 1964

3 Bruce D A Langfilt T W Miller J D Schutz H Vapalakti M Stanek A & Goldberg H I Regional cerebral blood flow intracranial pressure and brain metabolism in comatose patients *J Neurosurg 38* 131 1973

4 Chu J Clements J A Cotton E K Klaus M H Sweet A Y & Tolley W H Neonatal pulmonary ischemia Part I Clinical and physiological studies *Pediatrics 40* 709 1967

5 Courville C B Antenatal and paranatal circulatory disorders as a cause of cerebral damage in early life *J Neuropathol Exp Neurol 18* 115 1959

6 Dawes C S *Foetal and Neonatal Physiology* Year Book Medical Publishers Inc Chicago 1968 p 143

7 De Reuck J Chattha S A & Richardson E P Pathogenesis and evolution of periventricular leukomalacia in infancy *Arch Neurol 27* 229 1972

8 van der Eecken H *Anastomoses between the Leptomeningeal Arteries of the Brain* Ch C Thomas Springfield 1959

9 Elliott K A C & Jesper H H Physiological salt solutions for brain surgery Studies of local pH and pial vessels reactions to buffered and unbuffered isotonic solutions *J Neurosurg 6* 140 1949

10 Enevoldsen E & Jensen S T Autoregulation and CO_2 reactivity of cerebral blood flow in patients with severe head injury *J Neurosurg* in press

11 Fieschi C Bathistini N Bedusdir A Boselli L & Rossanda M Regional cerebral blood flow and intraventricular pressure in acute head injuries *J Neurol Neurosurg Psychiatry 37* 1378–1388 1974

12 Gartner L M Snyder R N Chabon R S & Bernstein J Kernicterus High incidence in pre

Acta Pædiatr Scand 68 65–69 1979

SERUM RETINOL BINDING PROTEIN AND VITAMIN A LEVELS IN MALNOURISHED CHILDREN

V REDDY M MOHANRAM and N RAGHURAMULU

From the National Institute of Nutrition Indian Council of Medical Research Jamai Osmania Hyderabad India

ABSTRACT Reddy V Mohanram M and Raghuramulu N (National Institute of Nutrition Hyderabad India) Serum retinol binding protein and vitamin A levels in malnourished children Acta Paediatr Scand 68 65 1979 —Serum levels of vitamin A and retinol binding protein (RBP) were measured in children with vitamin A deficiency in children with protein energy malnutrition (PEM) and in normal children before and after administration of 100 000 IU of water miscible vitamin A Serum vitamin A and RBP levels were significantly low in children with vitamin A deficiency and in children with severe PEM whereas the values in milder grades of PEM were similar to those of normal subjects In severely malnourished children with corneal lesions serum vitamin A concentration was reduced to a much greater extent than the level of serum RBP Administration of vitamin A resulted in a significant increase in serum levels of both the components within 4 hours in all the 3 groups of children The increase in RBP concentration observed in children with PEM was similar to that in vitamin A deficient children These results indicate that in malnourished children particularly in those who are at risk of developing keratomalacia vitamin A is the main limiting factor It is therefore recommended that children with PEM should be treated with vitamin A in addition to dietary protein and calories

KEY WORDS Vitamin A malnutrition retinol binding protein

Protein-energy malnutrition (PEM) and vitamin A deficiency are the two most frequently encountered deficiency diseases among children in many developing countries Ocular signs of vitamin A deficiency are seen in 30–40% of the children suffering from kwashiorkor (1) Levels of serum vitamin A are low in children with PEM whether or not they exhibit clinical signs of vitamin A deficiency (10) Recent studies suggest that retinol transport system is defective in this condition (13 15 16) Smith et al (13) reported that in children with kwashiorkor low serum vitamin A levels were associated with decreased concentration of retinol binding protein (RBP) and pre albumin Treatment with calories and protein but without supplemental vitamin A resulted in a significant increase in the concentration of all the three components It was therefore suggested that low levels of serum vitamin A may reflect a defective hepatic production of the carrier protein rather than vitamin A deficiency per se However some children with kwashiorkor have associated vitamin A deficiency and experiments in rats have shown that vitamin A status *per se* can modify RBP metabolism (6 7 9) Studies were therefore undertaken to examine the effect of vitamin A deficiency on the vitamin A transport system in malnourished children Serum RBP and vitamin A levels were measured in children with vitamin A deficiency and in children with PEM before and after administration of vitamin A

SUBJECTS AND METHODS

Ninety three children aged between 1–8 years were investigated They were classified into 4 groups based on their nutritional status

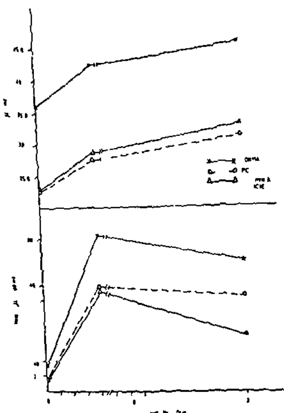


Fig 1 Effect of vitamin A administration on serum levels of vitamin A and RBP

and 39 9 μg/ml respectively The values observed in undernourished children (group II) were not different from those of normal children Serum levels of vitamin A and RBP were significantly lower in vitamin A deficient children (group III) and in children with PEM (group IV) as compared to normal subjects (group I) The mean concentrations were significantly lower in malnourished children with corneal lesions than in those without ocular signs (Table 1)

Following the administration of vitamin A there was a significant increase in serum vitamin A and RBP levels both at 4 hours and 24 hours in all the 3 groups of children studied However the percentage increase in serum RBP concentration was greater in malnourished children than in normals (Table 2) Response to vitamin A administration was similar in children with vitamin A deficiency and in children with PEM (Fig 1)

DISCUSSION

In the present study serum levels of vitamin A and RBP were found to be low in children with vitamin A deficiency (group III) and administration of vitamin A resulted in a significant increase in the concentration of both the components within 4 hours These observations are in line with those reported in rats (6 7 9) and suggest that the release of RBP from the liver depends on the availability of vitamin A Since these children did not have associated PEM of severe form synthesis of RBP may not be limited Following the massive dose of vitamin A the rise in serum vitamin A was much more marked than the rise in RBP suggesting that a part of the circulating vitamin was still in the ester form at 24 hours

In undernourished children serum levels of vitamin A and RBP were not different from those of normal subjects indicating that the vitamin transport system was not affected by mild degrees of PEM Whereas in children with severe PEM serum levels of both the components were significantly reduced—an observation similar to that reported by other workers (13 15 16) The regulation of serum vitamin A concentration in PEM seems to be influenced by protein nutrition as well as vitamin A status of the children Studies in Egyptian children with kwashiorkor have shown that treatment with protein and calories but without vitamin A resulted in increased levels of serum vitamin A and RBP after 2–4 weeks (15) These observations suggest that protein-calorie deficiency may influence vitamin A metabolism by interfering with hepatic synthesis of RBP In the studies reported here administration of vitamin A to children with kwashiorkor resulted in a rapid and significant increase in serum vitamin A as well as serum RBP within 4 hours before any change in protein nutrition could occur These results are similar to those observed in vitamin A defi

Table 1 *Serum vitamin A and RBP levels in children*
Values are mean ± S E

Groups	No of subjects	Serum albumin (g/100 ml)	Serum vitamin A (μg/100 ml)	Serum (RBP μg/ml)
I Normal	26	3 2±0 02	38 5±2 77	39 9±2 26
II Undernourished	12	2 8±0 08	31 4±4 15	34 5±3 46
III Vitamin A deficiency	13	2 7±0 19	17 7±2 36	22 8±1 77
IV Severe PEM				
(a) Without vitamin A deficiency signs	14	1 70±0 16	19 2±2 31	27 1±3 86
(b) With conjunctival lesions	11	1 30±0 19	17 3±3 55	27 2±3 61
(c) With corneal lesions	17	1 30±0 12*	6 1±0 85	19 6±1 0?

* $p<0.001$ as compared to group I

Group I 26 apparently normal children aged 2–8 years Their weights were above 80% of the local standard (3)

Group II 12 undernourished children aged 2–8 years Their weights were below 80% of the standard but they had no clinical signs of PEM

Group III 13 children aged 3–8 years who had ocular signs of vitamin A deficiency like conjunctival xerosis and/or bitot spots Their weights ranged from 70–90% of the standard and they had no clinical signs of PEM

Group IV 42 children with severe PEM aged 1–5 years Most of them were of marasmic kwashiorkor type showing oedema as well as emaciation 14 children had no clinical signs of vitamin A deficiency while 11 children had conjunctival xerosis and/or bitot spots (X_1 A and B) and 17 children had serious corneal lesions of X_2 and X_3 type of WHO nomenclature (17)

Fasting blood samples were collected from all the children initially Ten normal children of group I 13 vitamin A deficient children of group III and 20 children with PEM in group IV including all the 17 with corneal lesions were administered a single dose of 100 000 I U (30 000 μg) of water miscible vitamin A palmitate (aquesol) intramuscularly and blood samples were collected at 4 hours and 24 hours after the dose Serum vitamin A was determined in duplicate by a micro fluorometric method (11) The vitamin A values obtained by this method for malnourished children were in close agreement with the standard macro procedures (12) Serum RBP was assayed by immunodiffusion technique (5) the standard human RBP and human anti RBP were obtained from Behringwerke West Germany Serum albumin was determined by the dye method (2)

RESULTS

Undernourished children (group II) and vitamin A deficient children (group III) had slightly lower levels of serum albumin as compared to those of normal subjects Whereas the mean concentration of serum albumin was significantly decreased in children with severe PEM In normal children the mean levels of serum vitamin A and RBP were 38 5 μg/100 ml

Table 2 *Effect vitamin A administration*[a] *on serum vitamin A and RBP levels*
Values are mean ± S E

Group	No of subjects	Vitamin A μg/100 ml 0 hr	4 hr	24 hr	RBP μg/ml 0 hr	4 hr	24 hr
I Normal	10	33 8 ±4 08	205 0 ±62 75**	170 8 ±67 96**	36 5 ±3 38	42 2 ±3 70 **	45 6 ±4 10 **
II Vitamin A deficiency	13	17 8 ±6 89	131 6 ±98 27***	76 2 ±61 27 **	23 0 ±6 38	29 1 ±8 30	33 3 ±10 12
III Severe PEM	20	15 9 ±2 65	139 6 ±35 44 **	125 5 ±24 13***	22 8 ±2 24	27 7 ±2 35	31 4 ±2 78 *

[a] 100 000 I U vitamin A (Aquesol)
* $p<0.05$ ** $p<0.01$ *** $p<0.0001$ as compared to 0 hr

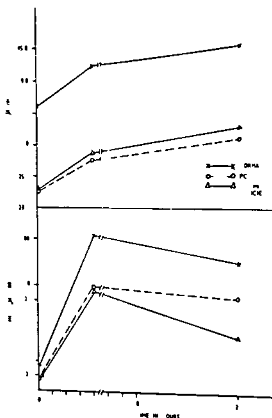


Fig 1 Effect of vitamin A administration on serum levels of vitamin A and RBP

and 39 9 μg/ml respectively The values observed in undernourished children (group II) were not different from those of normal children Serum levels of vitamin A and RBP were significantly lower in vitamin A deficient children (group III) and in children with PEM (group IV) as compared to normal subjects (group I) The mean concentrations were significantly lower in malnourished children with corneal lesions than in those without ocular signs (Table 1)

Following the administration of vitamin A there was a significant increase in serum vitamin A and RBP levels both at 4 hours and 24 hours in all the 3 groups of children studied However the percentage increase in serum RBP concentration was greater in malnourished children than in normals (Table 2) Response to vitamin A administration was similar in children with vitamin A deficiency and in children with PEM (Fig 1)

DISCUSSION

In the present study serum levels of vitamin A and RBP were found to be low in children with vitamin A deficiency (group III) and administration of vitamin A resulted in a significant increase in the concentration of both the components within 4 hours These observations are in line with those reported in rats (6 7 9) and suggest that the release of RBP from the liver depends on the availability of vitamin A Since these children did not have associated PEM of severe form synthesis of RBP may not be limited Following the massive dose of vitamin A the rise in serum vitamin A was much more marked than the rise in RBP suggesting that a part of the circulating vitamin was still in the ester form at 24 hours

In undernourished children serum levels of vitamin A and RBP were not different from those of normal subjects indicating that the vitamin transport system was not affected by mild degrees of PEM Whereas in children with severe PEM serum levels of both the components were significantly reduced—an observation similar to that reported by other workers (13 15 16) The regulation of serum vitamin A concentration in PEM seems to be influenced by protein nutrition as well as vitamin A status of the children Studies in Egyptian children with kwashiorkor have shown that treatment with protein and calories but without vitamin A resulted in increased levels of serum vitamin A and RBP after 2–4 weeks (15) These observations suggest that protein-calorie deficiency may influence vitamin A metabolism by interfering with hepatic synthesis of RBP In the studies reported here administration of vitamin A to children with kwashiorkor resulted in a rapid and significant increase in serum vitamin A as well as serum RBP within 4 hours before any change in protein nutrition could occur These results are similar to those observed in vitamin A defi

cient states and suggest that in PEM vitamin A is a limiting factor While protein deficiency may cause impaired synthesis of RBP vitamin A deficiency interferes with its release from the liver Thus deficiencies of both the nutrients seem to contribute to lowering of serum vitamin A concentration in children with PEM

Under physiological conditions retinol and RBP are present in serum in equimolar ratio and after retinol is taken up by the target tissues free RBP is metabolised by the kidney (8) In normal subjects only 15–20% of the total RBP is in the apo form (14) In severely malnourished children with corneal lesions studied here although serum vitamin A concentration was considerably lowered the RBP concentration did not fall to the same extent The ratio of RBP retinol was therefore raised indicating that vitamin A levels were reduced in spite of carrier protein being available It is possible that in severe vitamin A deficiency uptake of retinol by the depleted tissues may be very rapid resulting in a higher concentration of apo RBP in serum However this can be answered only by determining the levels of apo and holo RBP separately

Although the authors have not commented on it similar results have been obtained in children with PEM studied by Smith et al (13 15) A careful look at their data reveals that in children with marasmic kwashiorkor who had poor hepatic stores of vitamin A the concentration of serum vitamin A was reduced to a much greater extent than was the level of serum RBP suggesting that the ratio of holo to apo RBP was altered

These findings are of considerable significance since they indicate that in malnourished children particularly in those who are at risk of developing keratomalacia vitamin A is the main limiting factor In Egyptian and central American children with PEM although serum vitamin A levels were low none of them had serious corneal involvement like the Indian children studied here This difference can be explained on the basis of vitamin A status of the children PEM alone can lead to a moderate reduction in the serum concentration of both RBP and vitamin A but when there is associated vitamin A deficiency serum vitamin A concentration decreases further increasing the risk of keratomalacia Thus the role of vitamin A seems to be more critical than that of protein in the development of keratomalacia and blindness

Children with PEM should therefore be treated with vitamin A in addition to dietary protein and calories This is of utmost importance in case of corneal involvement Vitamin A deficiency can be corrected rapidly while improvement in protein nutrition is a slow process

A single injection of 100 000 I U (30 000 μg) of water miscible vitamin A will not only produce an increase in serum vitamin A concentration but will also result in rapid clinical improvement We have observed that early corneal xerosis clears up completely and vision is restored to normal within a few days after the dose but in more advanced cases of keratomalacia scar formation is inevitable Oral administration of vitamin A has also been found to be effective but parenteral administration is preferable since a majority of children with PEM have associated gastroenteritis

Malnourished children admitted to the hospital were given vitamin A in addition to dietary protein and calories Though this is the best method of treatment some children with PEM and corneal xerosis were treated as out patients because their parents refused admission It was observed that administration of vitamin A alone resulted in complete reversal of corneal xerosis The vision could be saved though there was no improvement in the general condition These observations confirm the above hypothesis and have a bearing on the treatment of this condition

ACKNOWLEDGEMENT

We wish to thank Dr S G Srikantia Director National Institute of Nutrition Hyderabad for helpful discussion

and suggestions The skillful technical assistance provided by Mr M Chandrasekharan and Mr S Chennaiah is acknowledged

REFERENCES

1 Chandra H Venkatachalam P S Belavady B Reddy V & Gopalan C Some observations on vitamin A deficiency in Indian children *Indian J Child Health* 9 589 1960

2 Doumas B T Watson W A & Biggs H G Albumin standards and the measurement of serum albumin with bromocresol green *Clin Chim Acta* 31 87 1971

3 Indian Council of Medical Research (ICMR) Growth and physical development of Indian infants and children *Tech Rep Series* No 18 1972

4 Ingenbleek Y Schrieck H G V DeNayer P & Devisscher M The role of retinol binding protein in protein-calorie malnutrition *Metabolism* 24 633 1975

5 Mancini G Carbonara A O & Heremans J F Immunochemical quantitation of antigens by single radial immunodiffusion *Int J Immunochem* 2 235 1965

6 Muhilal H & Glover J Effect of dietary deficiencies of protein and retinol on the plasma retinol binding protein in the rat *Br J Nutr* 32 549 1974

7 Muto Y Smith J E Milch P O & Goodman D S Regulation of retinol binding protein metabolism by vitamin A status in the rat *J Biol Chem* 247 2542 1972

8 Peterson P A Nilsson S F Östberg L Rask L & Vahlquist A Aspects of the metabolism of retinol binding protein and retinol *Vitam Horm* 32 181 1974

9 Peterson P A Rask L Östberg L Andersson L Kemwendo F & Pertoft H Studies on the transport and cellular distribution of vitamin A in normal and vitamin A deficient rats with special reference to the vitamin binding plasma protein *J Biol Chem* 248 4009 1973

10 Reddy V & Srikantia S G Serum vitamin A in kwashiorkor *Am J Clin Nutr* 18 105 1966

11 Selvaraj R J & Susheela T P Estimation of serum vitamin A by a microfluorometric procedure *Clin Chim Acta* 27 165 1970

12 Sivakumar B Evaluation of a microspectrofluorometric method for serum vitamin A *Clin Chim Acta* 79 189 1977

13 Smith F R Goodman D S Arroyave G & Viteri F Serum vitamin A retinol binding protein and prealbumin concentrations in protein-calorie malnutrition II Treatment including supplemental vitamin A *Am J Clin Nutr* 26 982 1973

14 Smith F R Raz A & Goodman D S Radioimmunoassay of human plasma retinol binding protein *J Clin Invest* 49 1754 1970

15 Smith F R Goodman D S Zaklama M S Gabr M K Maraghy S E & Patwardhan V N Serum vitamin A retinol binding protein and prealbumin concentration in protein calorie malnutrition I A functional defect in hepatic retinol release *Am J Clin Nutr* 26 973 1973

16 Venkataswamy G Glover J Cobby M & Pirie A Retinol binding protein in serum of xerophthalmic malnourished children before and after treatment at a nutrition centre *Am J Clin Nutr* 30 1968 1977

17 Vitamin A deficiency and xerophthalmia W H O *Tech Rep Series No 590 1976*

Submitted Febr 16 1978
Accepted May 11 1978

(V R) National Institute of Nutrition
Indian Council of Medical Research
Jamai-Osmania (P O)
Hyderabad 500007 A P
India

FINGERPRINTS IN CONGENITAL RUBELLA FOLLOWING MATERNAL GAMMA GLOBULIN

L J ROSS

From the Department of Pediatrics New York University Medical Center New York U S A

ABSTRACT Ross L J (Department of Pediatrics New York University Medical Center New York U.S A) Fingerprints in congenital rubella following maternal gamma globulin Acta Paediatr Scand 68 71 1979 —The fingertip skin ridge patterns of ten children with the congenital rubella syndrome whose mothers had received gamma globulin following exposure to german measles during pregnancy were compared with those of 29 patients whose mothers had not received gamma globulin and with those of 162 control children Statistical evaluation of the pattern profiles by the Mann Whitney U test showed that the dermatoglyphics of the two groups of patients differed significantly from each other Each group also differed from the control children there was a significant ($p<0.001$) increase in whorls in patients whose mothers had not had gamma globulin but an increase ($p=0.001$) in ulnar loops in those whose mothers had received gamma globulin

KEY WORDS Dermatoglyphics congenital rubella gamma globulin

Patterns of palmar skin ridging are believed to attain their definitive configuration by the 19th week of fetal life (4) A fingertip pattern which can be considered abnormal is very rarely seen in an individual patient but the altered relative distribution of the four main pattern types (whorls ulnar loops radial loops and arches) observed in certain groups of patients (6) presumably is a result of deviant embryologic development

An increased percent of fingertips with whorl patterns has been observed in patients with the congenital rubella syndrome (1 7 8 9) as has an increased frequency of individuals with whorls on eight nine or ten finger tips (3 7 9) Our finding of a paucity of whorls in a small group of patients whose mothers had gestational rubella after having been given prophylactic gamma globulin prompted the present report

CLINICAL MATERIAL AND METHODS

The fingertip skin ridge patterns of 39 children who had anomalies compatible with the congenital rubella syndrome were studied All patients were born in the New York City area and the majority attended the Rubella Birth Defect Evaluation Project of New York University *Medical Center* Rubella virus was isolated and/or there was serologic confirmation of the diagnosis in 7 instances There was a physician s prospective clinical diagnosis of gestational rubella for eight of the twelve mothers of the remaining children

The mothers of ten patients received gamma globulin (preparations unknown) because of exposure to rubella during pregnancy all ten mothers nevertheless subsequently developed a rash Their children will be referred to as having congenital rubella after gamma globulin the other 29 patients as having congenital rubella (without prior gamma globulin) Cardiovascular anomalies hearing impairment mental retardation and neonatal purpura were present in both groups of patients but cataracts birth weights below 2 270 g glaucoma and bone lesions were noted only in the children with congenital rubella (without prior gamma globulin)

The first four children in Table 1 were born in October 1955–January 1956 patient 19 in December 1963 patient 25 in January 1964 and the rest from July 1964–March 1965 Patients 1–16 and 30–33 were female Patients 11 12 23 and 24 were Negro patients 13–16 and 25–29 were Puerto Rican and the rest (including all in Table 2) were white

A group of control children free of congenital anomalies was drawn from well baby and general pediatric clinics and a nursery school in New York City There were 70 boys and 92 girls Forty two children were white 37 Negro and 83 Puerto Rican The birth weight of 35 children was under 2.7 kg

Table 1 *Fingertip skin ridge patterns of those children with congenital rubella whose mothers did not receive gamma globulin before developing gestational rubella*

W=whorl U=ulnar loop R=radial loop A=arch *= no recognizable pattern X=poor print prevented analysis of pattern

	Fingertip patterns	
Pt	Right hand fingers 5 4 3 2 1	Left hand fingers 5 4 3 2 1
1	WWUWU	WWUWU
2	UUUWX	UUURU
3	WUUUU	WWWUU
4	UWWWU	UWWUU
5	WWUWU	WWWWW
6	WWURW	WUUWW
7	UWUWU	UWUWW
8	UUUWU	UUUUU
9	UWUWW	WWUWW
10	UWUUU	UWUUU
11	WWWWW	WWWWW
12	UUAUU	UUURU
13	WWUWW	WUUWW
14	UWUUU	WWUUU
15	WWWWW	WWWWW
16	UWWUW	WWUUU
17	WWUWW	WWUUW
18	WWWWW	UWWWW
19	WWWWW	WWWWW
20	WWWWU	UWWWU
21	WWUUU	UWUUU
22	UWU U	UWU U
23	WWWWW	WWWWW
24	WWWUW	WWWWW
25	UWUUU	UUUUU
26	WWUUU	WWUUU
27	UWUWW	UWUUW
28	WUUUU	WWWUU
29	UUUUU	UUUUU

Fingerprints of the four children damaged in the 1955 epidemic were recorded the skin of the fingertips of the remaining patients and of the control children was inspected through a 7×magnifying glass All dermatoglyphic observations were made by one person

The numbers of children with 0 1 2 10 whorls (hereafter referred to as the whorl profile) were determined as were the numbers of children with 0 1 2 10 ulnar loops (ulnar loop profile) the numbers with 0 1 2 10 arches (arch profile) and the numbers with 0 1 2 and 3 radial loops (radial loop profile) Each of the four pattern profiles of the children with congenital rubella after gamma globulin was compared with the corresponding profile of the children with congenital rubella (without prior gamma globulin) and the pattern profiles of each group of patients were compared with those of the control children Inter group differences were evaluated statistically by the Mann Whitney U test

RESULTS

The ridge patterns of the patients are recorded in detail in Tables 1 and 2 The percentage distribution of pattern types on the fingertips of control children and patients is presented in Table 3 The frequencies of whorls and ulnar loops in both groups of patients with *congenital rubella* are outside the wide ranges of frequencies of these patterns observed in various Caucasian and Negro population samples (5)

Whorl and ulnar loop histogram profiles (7) of patients and control children are shown in Fig 1 The statistically significant inter group differences in pattern profiles were

a) Congenital rubella (without prior gamma globulin) compared with control children whorl profile ($p<0\ 001$) arch profile ($p<0\ 01$) and ulnar loop profile ($p<0\ 02$)

b) Congenital rubella after gamma globulin compared with control children ulnar loop profile ($p=0\ 001$) and whorl profile ($p=0\ 01$) (Data limited to the white children showed a significant ($p<0\ 01$) difference in ulnar loop and whorl profiles)

c) Congenital rubella after gamma globulin compared with congenital rubella (without prior gamma globulin) whorl profile ($p<0\ 001$) ulnar loop profile ($p<0\ 001$) and arch profile ($p=0\ 02$) (Data limited to the white children showed a significant ($p<0\ 001$) difference in whorl and ulnar loop profiles)

DISCUSSION

In the past statistical evaluation of differences in the fingertip dermatoglyphics of two groups of individuals has usually been based on their pattern frequencies which in turn depend on the total numbers of fingertips with whorls with ulnar loops with radial loops and with arches This method has been challenged by Berka McClure Sonley & Thompson (2) and by Preus & Fraser (6) because it assumes that the pattern on each fingertip of an individual is independently determined Thompson &

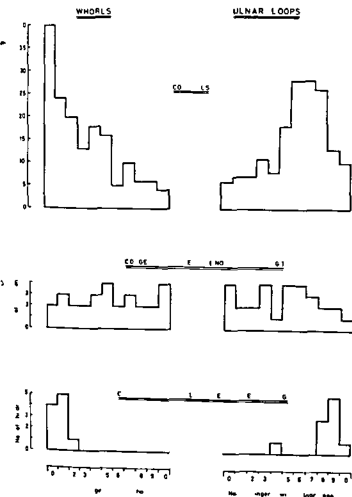


Fig 1 Whorl and ulnar loop histogram profiles of patients with congenital rubella and control children

Table 2 *Fingertip patterns of those children with congenital rubella whose mothers received gamma globulin before developing gestational rubella*

W=whorl U=ulnar loop R=radial loop A=arch

Pt	Gamma globulin			Fingertip patterns	
	Days after LMP	Amount (cc)	Days before rash	Right hand fingers 5 4 3 2 1	Left hand fingers 5 4 3 2 1
30	?	?	14	UUUUW	UUUUU
31	37	?	1?	UUUUU	UUARU
3?	40	11	7	UUUAU	UUUAU
33	57	?0	7	UUARW	UUAAW
34	?	?	16	UUUUW	UUUUU
35	31	?	14	UUUUU	UUURU
36	33	20	6	UUUUU	UUUUW
37	68 and 86	10 and 10	24 and 6	UWUUU	UUUUU
38	75	16	15	UWURU	UUUUU
39	77	?0	2	UUUUU	UUUUU

Table 3 *Frequencies of pattern types on the fingertips of control children and those with congenital rubella*

	Control children	Children with con genital rubella — Mothers received gamma globulin before developing rubella: No	Yes
Number of children	162	29	10
Percent of fingertips with			
Whorls	31	52	7
Ulnar loops	60	46	83
Arches	7	0 3	6
Radial loops	2	1	4
Unknown		1	

Bandler (10) have demonstrated mathematically that this can not be so and that the patterns in any one individual tend to be alike We suggest that it is more valid to compare the pattern profiles than the pattern frequencies of two groups since an individual is represented only once in a given pattern profile

Comparison of the whorl profile of our patients (without prior gamma globulin) with that of the controls showed that the increase in whorls in these patients was indeed highly significant ($p<0\ 001$) corroborating prior observations

However the patients whose mothers had received gamma globulin before developing gestational rubella had significantly fewer whorls and more ulnar loops than the children with congenital rubella (without prior gamma globulin) and unexpectedly than the control children as well The findings suggest that gamma globulin either alone or in interaction with rubella virus influenced fetal fingertip skin development

REFERENCES

1 Alter M & Schulenberg R Dermatoglyphics in the rubella syndrome *JAMA 197* 685 1966
2 Berka L McClure P D Sonley M J & Thompson M W Dermatoglyphics in childhood leukemia *Can Med Assoc J 105* 476 1971
3 Gumpel S M Clinical and social status of patients with congenital rubella *Arch Dis Child 47* 330 1972
4 Hale A R Breadth of epidermal ridges in the human fetus and its relation to the growth of the hand and foot *Anat Rec 105* 763 1949
5 Plato C C Cereghino J J & Steinberg F S The dermatoglyphics of American Caucasians *Am J Phys Anthrop 42* 195 1975
6 Preus M & Fraser F C Dermatoglyphics and syndromes *Am J Dis Child 124* 933 1972
7 Purvis Smith S G & Menser M A Dermatoglyphics in adults with congenital rubella *Lancet II* 141 1968
8 Purvis Smith S G & Menser M A Genetic and environmental influences on digital dermatoglyphics in congenital rubella *Pediatr Res 7* 215 1973
9 Purvis Smith S G Howard P R & Menser M A Dermatoglyphic defects and rubella teratogenesis *JAMA 209* 1865 1969
10 Thompson M S & Bandler E Finger pattern combinations in normal individuals and in Down s syndrome *Hum Biol 45* 563 1973

Submitted March 16 1978
Accepted May 25 1978

Kupat Holim
Ir Ganim
Jerusalem
Israel

Acta Pædiatr Scand 68 75–79 1979

RENAL FUNCTION IN INFANTS WITH HYPERBII IRUBINEMIA

U BROBERGER and A APERIA

From the Departments of Paediatrics at Karolinska Sjukhuset and St Goran s Child en s Hospital Stockholm Sweden

ABSTRACT Broberger U & Aperia A (Departments of Paediatrics at Karolinska Sjukhuset and St Goran s Children s Hospital Stockholm Sweden) Renal function in in fants with hyperbilirubinemia Acta Paediatr Scand 68 1979 —A total of 45 infants were studied on the fourth or fifth day of life 13 term and 10 pre term infants with serum bilirubin levels ranging between 257 and 390 μmol/l were compared with 12 term and 10 pre-term infants with serum bilirubin levels below 195 μmol/l The groups did not differ with regard to mean gestational age or mean post natal age GFR and C_{PAH} were determined with the single injection clearance method and ability to excrete Na was determined following an oral load ing of sodium chloride GFR was lower in infants with hyperbilirubinemia and correlated neg atively to the highest recorded serum bilirubin value C_{PAH} was similar in hyperbilirubinemic infants and controls The urinary sodium exretion was significantly higher in infants with hyperbilirubinemia

KEY WORDS GFR C_{PAH} urinary sodium excretion pre term full term hyper bilirubinemia

Depressed renal function in the newborn in fant is a normal state The glomerular filtra tion rate is 25–30% of that in children and adults The tubular capacity is also low Hence diseases that do not primarily affect the kid ney may still interfere with kidney function This has been reported in infants with the idiopathic respiratory distress syndrome (3 7) In adults and animals with hyperbilirubinemia depressed renal function has been reported (1 10 12 14 16) The present study was undertaken to investigate the effect of uncon jugated hyperbilirubinemia on glomerular fil tration and sodium homeostasis in term and pre term newborn infants

MATERIALS

A total of 45 infants were studied on the fourth or fifth day of life 13 term and 10 pre term infants with hyperbili rubinemia were compared with 12 term and 10 pre term infants without hyperbilirubinemia The gestational age of the pre term infants ranged between 33 and 36 weeks The weights and lengths of the infants corresponded to their gestational ages All pregnancies were uneventful Five infants were delivered by caesarean section 3 because of breech presentation (one pre term with and 2 without hy perbilirubinemia) and 2 because of narrow pelvis (one term with and one without hyperbilirubinemia) Apgar scores were 7 or more at 1 min and 8 or more at 5 min of age in all infants studied The highest recorded serum bili rubin levels ranged between 260 and 390 μmol/l in term infants and between 257 and 376 μmol/l in pre term in fants Conjugated bilirubin levels did not exceed 20 μmol/l In term and pre term controls the serum bilirubin was below 195 μmol/l The clinical findings in term and pre term infants with hyperbilirubinemia are shown in Table I

All infants re eived breast milk during the studies in an amount of 100–120 ml/kg B W /24 hrs The pre term in fants were kept in Isolette incubators The infants were disturbed as little as possible during the studies and showed no signs of discomfort Informed parental consent was obtained in all cases The protocol was approved by the Committee of Ethics at Karolinska Institute

METHODS

Gestational age was determined with the method de scribed by Dubowitz et al (5) Total serum bilirubin was determined in duplicate with a Bilirubinometer (American Optical Corp) Conjugated bilirubin was determined with Nosslin s method (11) Sodium in urine was analysed by a flame photometer (Eppendorff) Hematocrit was esti mated in glass capillaries rinsed with heparin and centri fuged for 5 min at 12 000 g

Table 3 *Frequencies of pattern types on the fingertips of control children and those with congenital rubella*

	Control children	Children with congenital rubella — Mothers received gamma globulin before developing rubella: No	Yes
Number of children	162	29	10
Percent of fingertips with			
Whorls	31	52	7
Ulnar loops	60	46	83
Arches	7	0 3	6
Radial loops	2	1	4
Unknown		1	

Bandler (10) have demonstrated mathematically that this can not be so and that the patterns in any one individual tend to be alike We suggest that it is more valid to compare the pattern profiles than the pattern frequencies of two groups since an individual is represented only once in a given pattern profile

Comparison of the whorl profile of our patients (without prior gamma globulin) with that of the controls showed that the increase in whorls in these patients was indeed highly significant ($p<0\ 001$) corroborating prior observations

However the patients whose mothers had received gamma globulin before developing gestational rubella had significantly fewer whorls and more ulnar loops than the children with congenital rubella (without prior gamm globulin) and, unexpectedly than the contr children as well The findings suggest th gamma globulin either alone or in interacti with rubella virus influenced fetal fingert skin development

REFERENCES

1 Alter M & Schulenberg R Dermatoglyphics the rubella syndrome *JAMA 197* 685 1966
2 Berka L McClure P D Sonley M J & Tho son M W *Dermatoglyphics in childhood leuke Can Med Assoc J 105* 476 1971
3 Gumpel S M Clinical and social status of tients with congenital rubella *Arch Dis Child* 330 1972
4 Hale A R Breadth of epidermal ridges in human fetus and its relation to the growth of hand and foot *Anat Rec 105* 763 1949
5 Plato C C Cereghino J J & Steinberg F The dermatoglyphics of American Caucasians *J Phys Anthrop 42* 195 1975
6 Preus M & Fraser F C Dermatoglyphics syndromes *Am J Dis Child 124* 933 197?
7 Purvis Smith S G & Menser M A Derm glyphics in adults with congenital rubella *Lan II* 141 1968
8 Purvis Smith S G & Menser M A Genetic environmental influences on digital dermatoglyp in congenital rubella *Pediatr Res 7* 215 1973
9 Purvis Smith S G Howard P R & Menser A Dermatoglyphic defects and rubella teratogene *JAMA 209* 1865 1969
10 Thompson M S & Bandler E Finger patt combinations in normal individuals and in Dow syndrome *Hum Biol 45* 563 1973

Submitted March 16 1978
Accepted May 25 1978

Kupat Holim
Ir Ganim
Jerusalem
Israel

Table 3 *C_{PAH} gestational age post natal age and hematocrit in infants with hyperbilirubinemia and controls*

	Full terms		Pre terms	
	Hyperbil	Control	Hyperbil	Control
C_{PAH} (ml/1 73 m B S /min)	190 1±18 7	192 0±46 0	117 0±36 0	1[illegible]9 3±25 4
Gestational age (weeks)	39 0± 0 0	40 8± 0 8	35 0± 0 0	34 4± 1 8
Post natal age (hrs)	13[illegible] 7±38 7	103 8±11 7	10[illegible] 7±23 9	132 4±24 7
Hematocrit (%)	49 7± 2 1	48 0± 3 8	6[illegible] 3± 2 1	41 [illegible]± 6 7
n	3	5	3	5

Values are means±S D

after administration of sodium chloride The urinary sodium excretion was calculated as the average hourly excretion per 1 73 m² B S Inulin in blood was determined using the Anthron method (8) PAH in blood was determined using the method described by Smith et al (17)

RESULTS

The GFR C_{PAH} and sodium excretion in infants with hyperbilirubinemia and in controls are summarized in Tables 2 3 and 4 respectively The gestational age post natal age and hematocrit in the different groups are also shown

Glomerular filtration rate

GFR was determined in 9 term infants with hyperbilirubinemia and 5 controls of the same post natal age and hematocrit GFR was significantly lower in infants with hyperbilirubinemia i e 36 0 vis a vis 53 2 ml/1 73 m² B S /min in the controls (0 0025>p>0 0005)

GFR was also determined in 6 pre term infants with hyperbilirubinemia and in 5 controls Here the hyperbilirubinemic infants also had lower figures for GFR 31 1 vis a vis 38 3 ml/1 73 m² B S /min although the difference was not significant (0 10>p>0 05) There was a significant negative correlation between GFR and the highest serum bilirubin value recorded before the clearance study r=−0 48 (0 05>p>0 025) See Fig 1

Clearance of PAH

C_{PAH} was determined in 3 term and 3 pre term infants with hyperbilirubinemia and compared with 5 controls of similar gestational age and post natal age No difference was found between hyperbilirubinemic infants and controls The figures for term infants were 190 1 and 192 0 ml/1 73 m² B S /min respectively and for pre term infants 117 0 and 129 0 ml/1 73 m² B S /min

Average hourly sodium excretion after an oral sodium load (UNaV)

UNaV was studied in 5 term infants with hyperbilirubinemia and 7 controls UNaV was

Table 4 *Average hourly sodium excretion UNaV gestational age post natal age and hematocrit in infants with hyperbilirubinemia and controls*

	Full terms		Pre terms	
	Hyperbil	Control	Hyperbil	Control
UNaV (mmol/1 73 m² B S /h)	4 4± [illegible] 1	2 1± 1 9	3 2± 1 0	2 0± 0 5
Gestational age (weeks)	39 8± 0 5	40 0± 1 0	35 [illegible]± 0 8	34 4± 1 1
Post natal age (hrs)	136 2±[illegible]3 4	116 6±29 2	109 7±19 9	126 6±26 0
Hematocrit (%)	53 [illegible]± 5 8	58 0± 7 3	53 [illegible]± 8 0	53 2± 4 4
n	5	7	6	5

Values are means±S D

Table 1 *Clinical data and post natal age at investigation in full term and pre term infants with hyperbilirubinemia*

Pat no	Sex	Gest age (weeks)	Birth weight (g)	Diagnosis	Serum bil µmol/l/ age hrs	Photo-therapy age hrs	Age at invest (hrs)
Full terms							
1	F	39	3 190	Phys jaund	308/ 88	72–120	155
2	M	39	2 950	ABO incomp	316/155	120–168	155
3	F	40	4 000	Phys jaund	387/ 86	73– 81	86
4	F	38	3 190	Phys jaund	351/100	84– 90	107
5	F	39	3 680	Phys jaund	356/100	40–134	110
6	F	39	2 700	ABO incomp	284/ 87	60–130	111
7	M	40	3 910	ABO incomp	308/ 71	77–100	107
8	M	40	3 400	ABO incomp	282/111	42– 80	111
9	F	42	3 250	Phys jaund	260/ 85	85– 99	109
10	F	40	2 940	Phys jaund	380/ 72	72–163	144
11	M	40	3 600	Phys jaund	375/ 75	78– 93	100
12	M	40	3 100	ABO incomp	390/143	126–170	155
13	M	40	3 650	Phys jaund	340/102	60–120	176
Pre terms							
1	M	34	2 270	Phys jaund	257/ 81	84–102	113
2	F	36	2 760	ABO incomp	376/ 75	77–150	134
3	F	35	2 100	Phys jaund	316/ 95	48–169	101
4	M	36	2 850	ABO incomp	333/104	100–170	117
5	M	35	2 790	ABO incomp	328/122	127–139	127
6	F	35	2 390	ABO incomp	342/ 76	18–144	76
7	F	36	2 680	ABO incomp	311/110	89–132	110
8	M	34	2 000	Phys jaund	354/ 88	85– 99	112
9	F	36	2 550	Phys jaund	272/120	104–140	144
10	M	36	2 890	ABO incomp	345/157	96–192	181

Single injection inulin and PAH clearance Inulin and PAH were injected i v in a dose of 100 and 15 mg/kg B W respectively Calibrated glass syringes were used Samples of the test solutions were kept for determination of inulin and PAH concentrations Blood (0 4 ml) was collected before the test solutions were given and then every third minute from 7 to 19 min and every fifth minute from 45 to 70 or 90 min The samples were collected in small test tubes rinsed with heparin and were kept on ice until they were centrifuged The plasma was frozen and stored until analysed The graphic resolution of the experimental curve was performed using the method described by Sapirstein et al (15) The procedure of single injection inulin clearance and its validity has been described in detail elsewhere (2)

The renal excretion of an oral sodium load (UNaV) was studied during standardized fluid expansion Breast milk diluted with 50% water was administered by gastric tube in an amount corresponding to 2% of body weight during the first hour and then in an amount of 0 5% every 30 min When a stable diuresis was established 50–60 mmol NaCl/1 73 m² B S (2–3 mmol/kg B W) was given orally in diluted breast milk Urine was collected after spontaneous voiding in urinary bags for a time period of 4–6 hrs

Table 2 *GFR gestational age post natal age and hematocrit in infants with hyperbilirubinemia and controls*

	Full terms		Pre terms	
	Hyperbil	Control	Hyperbil	Control
GFR (ml/1 73 m² B S /min)	36 0±7 3	53 2±11 1	31 1± 6 3	38 3± 8 1
Gestational age (weeks)	39 6± 1 1	40 8± 0 8	35 0± 0 6	34 4± 1 8
Post natal age (hrs)	116 2±23 3	103 8±11 7	124 2±35 5	132 4±24 7
Hematocrit (%)	49 1± 4 9	48 0± 3 8	57 7± 5 5	41 2± 6 7
n	9	5	6	5

Values are means±S D

Table 3 C_{PAH} *gestational age post natal age and hematocrit in infants with hyperbilirubinemia and controls*

	Full terms		Pre terms	
	Hyperbil	Control	Hyperbil	Control
C_{PAH} (ml/1 73 m² B S /min)	190 1±18 7	19? 0±46 0	117 0±36 0	129 3±25 4
Gestational age (weeks)	39 0± 0 0	40 8± 0 8	35 0± 0 0	34 4± 1 8
Post natal age (hrs)	132 7±38 7	103 8±11 7	10? 7±23 9	132 4±24 7
Hematocrit (%)	49 7± 2 1	48 0± 3 8	6? 3± 2 1	41 2± 6 7
n	3	5	3	5

Values are means±S D

after administration of sodium chloride The urinary sodium excretion was calculated as the average hourly excretion per 1 73 m² B S *Inulin in blood was determined* using the Anthron method (8) PAH in blood was determined using the method described by Smith et al (17)

RESULTS

The GFR C_{PAH} and sodium excretion in infants with hyperbilirubinemia and in controls are summarized in Tables 2 3 *and 4* respectively The gestational age post natal age and hematocrit in the different groups are also shown

Glomerular filtration rate

GFR was determined in 9 term infants with hyperbilirubinemia and 5 controls of the same post natal age and hematocrit *GFR was significantly* lower in infants with hyperbilirubinemia i e 36 0 vis a vis 53 2 ml/1 73 m² B S /min in the controls (0 0025>p>0 0005)

GFR was also determined in 6 pre term infants with hyperbilirubinemia and in 5 controls Here the hyperbilirubinemic infants also had lower figures for GFR 31 1 vis a vis 38 3 ml/1 73 m² B S /min although the *difference* was not significant *(0 10>p>0 05) There* was a significant negative correlation between GFR and the highest serum bilirubin value recorded before the clearance study $r=-0$ 48 *(0 05>p>0 025) See Fig 1*

Clearance of PAH

C_{PAH} was determined in 3 term and 3 pre term infants with hyperbilirubinemia and compared with 5 controls of similar gestational age and post natal age No difference was found between hyperbilirubinemic infants and controls The figures for term infants were 190 1 and 192 0 ml/1 73 m² B S /min respectively and for pre term infants 117 0 and 129 0 ml/1 73 m² B S /min

Average hourly sodium excretion after an oral sodium load (UNaV)

UNaV was studied in 5 term infants with *hyperbilirubinemia* and 7 controls UNaV was

Table 4 *Average hourly sodium excretion UNaV gestational age post natal age and hematocrit in infants with hyperbilirubinemia and controls*

	Full terms		Pre terms	
	Hyperbil	Control	Hyperbil	Control
UNaV (mmol/1 73 m² B S /h)	4 4± 2 1	2 1± 1 9	3 2± 1 0	2 0± 0 5
Gestational age (weeks)	39 8± 0 5	40 0± 1 0	35 ?± 0 8	34 4± 1 1
Post natal age (hrs)	136 2±23 4	116 6±?9 7	109 ?±19 9	1 6 6±26 0
Hematocrit (%)	53 2± 5 8	58 0± 7 3	53 2± 8 0	53 2± 4 4
n	5	7	6	5

Values are means±S D

Table 1 *Clinical data and post natal age at investigation in full term and pre term infants with hyperbilirubinemia*

Pat no	Sex	Gest age (weeks)	Birth weight (g)	Diagnosis	Serum bil µmol/l/ age hrs	Photo-therapy age hrs	Age at invest (hrs)
Full terms							
1	F	39	3 190	Phys jaund	308/ 88	72–120	155
2	M	39	2 950	ABO incomp	316/155	120–168	155
3	F	40	4 000	Phys jaund	387/ 86	73– 81	86
4	F	38	3 190	Phys jaund	351/100	84– 90	107
5	F	39	3 680	Phys jaund	356/100	40–134	110
6	F	39	2 700	ABO incomp	284/ 87	60–130	111
7	M	40	3 910	ABO incomp	308/ 71	77–100	107
8	M	40	3 400	ABO incomp	282/111	42– 80	111
9	F	42	3 250	Phys jaund	260/ 85	85– 99	109
10	F	40	2 940	Phys jaund	380/ 72	72–163	144
11	M	40	3 600	Phys jaund	325/ 75	78– 93	100
12	M	40	3 100	ABO incomp	390/143	126–170	155
13	M	40	3 650	Phys jaund	340/102	60–170	176
Pre terms							
1	M	34	2 270	Phys jaund	257/ 81	84–107	113
2	F	36	2 760	ABO incomp	376/ 75	77–150	134
3	F	35	2 100	Phys jaund	316/ 95	48–169	101
4	M	36	2 850	ABO incomp	333/104	100–120	117
5	M	35	2 790	ABO incomp	328/122	127–139	177
6	F	35	2 390	ABO incomp	342/ 76	18–144	76
7	F	36	2 680	ABO incomp	311/110	89–132	110
8	M	34	2 000	Phys jaund	354/ 88	85– 99	112
9	F	36	2 550	Phys jaund	272/120	104–140	144
10	M	36	2 890	ABO incomp	345/157	96–192	181

Single injection inulin and PAH clearance Inulin and PAH were injected i v in a dose of 100 and 15 mg/kg B W respectively Calibrated glass syringes were used Samples of the test solutions were kept for determination of inulin and PAH concentrations Blood (0 4 ml) was collected before the test solutions were given and then every third minute from 7 to 19 min and every fifth minute from 45 to 70 or 90 min The samples were collected in small test tubes rinsed with heparin and were kept on ice until they were centrifuged The plasma was frozen and stored until analysed The graphic resolution of the experimental curve was performed using the method described by Sapirstein et al (15) The procedure of single injection inulin clearance and its validity has been described in detail elsewhere (2)

The renal excretion of an oral sodium load (UNaV) was studied during standardized fluid expansion Breast milk diluted with 50% water was administered by gastric tube in an amount corresponding to 2% of body weight during the first hour and then in an amount of 0 5% every 30 min When a stable diuresis was established 50–60 mmol NaCl/1 73 m² B S (2–3 mmol/kg B W) was given orally in diluted breast milk Urine was collected after spontaneous voiding in urinary bags for a time period of 4–6 hrs

Table 2 *GFR gestational age post natal age and hematocrit in infants with hyperbilirubinemia and controls*

	Full terms		Pre terms	
	Hyperbil	Control	Hyperbil	Control
GFR (ml/1 73 m² B S /min)	36 0±7 3	53 2±11 1	31 1± 6 3	38 3± 8 1
Gestational age (weeks)	39 6± 1 1	40 8± 0 8	35 0± 0 6	34 4± 1 8
Post natal age (hrs)	116 2±23 3	103 8±11 7	124 2±35 5	132 4±24 7
Hematocrit (%)	49 1± 4 9	48 0± 3 8	57 7± 5 5	41 2± 6 7
n	9	5	6	5

Values are means±S D

Table 3 C_{PAH} *gestational age post natal age and hematocrit in infants with hyperbilirubinemia and controls*

	Full terms		Pre terms	
	Hyperbil	Control	Hyperbil	Control
C_{AH} (ml/1 73 m² B S /min)	190 1±18 7	192 0±46 0	117 0±36 0	129 3±25 4
Gestational age (weeks)	39 0± 0 0	40 8± 0 8	35 0± 0 0	34 4± 1 8
Post natal age (hrs)	132 7±38 7	103 8±11 7	102 7±23 9	132 4±74 7
Hematocrit (%)	49 7± 2 1	48 0± 3 8	67 3± 2 1	41 2± 6 7
n	3	5	3	5

Values are means±S D

after administration of sodium chloride The urinary sodium excretion was calculated as the average hourly excretion per 1 73 m B S Inulin in blood was determined using the Anthron method (8) PAH in blood was determined using the method described by Smith et al (17)

RESULTS

The GFR C_{PAH} and sodium excretion in infants with hyperbilirubinemia and in controls are summarized in Tables 2 3 and 4 respectively The gestational age post natal age and hematocrit in the different groups are also shown

Glomerular filtration rate

GFR was determined in 9 term infants with hyperbilirubinemia and 5 controls of the same post natal age and hematocrit GFR was significantly lower in infants with hyperbilirubinemia i e 36 0 vis a vis 53 2 ml/1 73 m² B S /min in the controls ($0\ 0025>p>0\ 0005$)

GFR was also determined in 6 pre term infants with hyperbilirubinemia and in 5 controls Here the hyperbilirubinemic infants also had lower figures for GFR 31 1 vis à vis 38 3 ml/1 73 m² B S /min although the difference was not significant ($0\ 10>p>0\ 05$) There was a significant negative correlation between GFR and the highest serum bilirubin value recorded before the clearance study $r=-0\ 48$ ($0\ 05>p>0\ 025$) See Fig 1

Clearance of PAH

C_{PAH} was determined in 3 term and 3 pre term infants with hyperbilirubinemia and compared with 5 controls of similar gestational age and post natal age No difference was found between hyperbilirubinemic infants and controls The figures for term infants were 190 1 and 192 0 ml/1 73 m² B S /min respectively and for pre term infants 117 0 and 129 0 ml/1 73 m² B S /min

Average hourly sodium excretion after an oral sodium load (UNaV)

UNaV was studied in 5 term infants with hyperbilirubinemia and 7 controls UNaV was

Table 4 *Average hourly sodium excretion UNaV gestational age post natal age and hematocrit in infants with hyperbilirubinemia and controls*

	Full terms		Pre terms	
	Hyperbil	Control	Hyperbil	Control
UNaV (mmol/1 73 m B S /h)	4 4± 2 1	2 1± 1 9	3 7± 1 0	2 0± 0 5
Gestational age (weeks)	39 8± 0 5	40 0± 1 0	35 2± 0 8	34 4± 1 1
Post natal ag (hrs)	136 2±73 4	116 6±29 2	109 7±19 9	126 6±26 0
Hematocrit (%)	53 2± 5 8	58 0± 7 3	53 2± 8 0	53 2± 4 4
n	5	7	6	5

Values are means±S D

Table 1 *Clinical data and post natal age at investigation in full term and pre term infants with hyperbilirubinemia*

Pat no	Sex	Gest age (weeks)	Birth weight (g)	Diagnosis	Serum bil µmol/l/ age hrs	Photo-therapy age hrs	Age at invest (hrs)
Full terms							
1	F	39	3 190	Phys jaund	308/ 88	72–120	155
2	M	39	2 950	ABO incomp	316/155	120–168	155
3	F	40	4 000	Phys jaund	387/ 86	73– 81	86
4	F	38	3 190	Phys jaund	351/100	84– 90	107
5	F	39	3 680	Phys jaund	356/100	40–134	110
6	F	39	2 700	ABO incomp	284/ 87	60–130	111
7	M	40	3 910	ABO incomp	308/ 71	77–100	107
8	M	40	3 400	ABO incomp	282/111	42– 80	111
9	F	42	3 250	Phys jaund	260/ 85	85– 99	109
10	F	40	2 940	Phys jaund	380/ 72	72–163	144
11	M	40	3 600	Phys jaund	325/ 75	78– 93	100
12	M	40	3 100	ABO incomp	390/143	126–170	155
13	M	40	3 650	Phys jaund	340/102	60–120	176
Pre terms							
1	M	34	2 270	Phys jaund	257/ 81	84–102	113
2	F	36	2 760	ABO incomp	376/ 75	77–150	134
3	F	35	2 100	Phys jaund	316/ 95	48–169	101
4	M	36	2 850	ABO incomp	333/104	100–170	117
5	M	35	2 790	ABO incomp	328/122	127–139	177
6	F	35	2 390	ABO incomp	342/ 76	18–144	76
7	F	36	2 680	ABO incomp	311/110	89–132	110
8	M	34	2 000	Phys jaund	354/ 88	85– 99	117
9	F	36	2 550	Phys jaund	272/120	104–140	144
10	M	36	2 890	ABO incomp	345/157	96–192	181

Single injection inulin and PAH clearance Inulin and PAH were injected i v in a dose of 100 and 15 mg/kg B W respectively Calibrated glass syringes were used Samples of the test solutions were kept for determination of inulin and PAH concentrations Blood (0 4 ml) was collected before the test solutions were given and then every third minute from 7 to 19 min and every fifth minute from 45 to 70 or 90 min The samples were collected in small test tubes rinsed with heparin and were kept on ice until they were centrifuged The plasma was frozen and stored until analysed The graphic resolution of the experimental curve was performed using the method described by Sapirstein et al (15) The procedure of single injection inulin clearance and its validity has been described in detail elsewhere (2)

The renal excretion of an oral sodium load (UNaV) was studied during standardized fluid expansion Breast milk diluted with 50% water was administered by gastric tube in an amount corresponding to 2% of body weight during the first hour and then in an amount of 0 5% every 30 min When a stable diuresis was established 50–60 mmol NaCl/1 73 m² B S (2–3 mmol/kg B W) was given orally in diluted breast milk Urine was collected after spontaneous voiding in urinary bags for a time period of 4–6 hrs

Table 2 *GFR gestational age post natal age and hematocrit in infants with hyperbilirubinemia and controls*

	Full terms		Pre terms	
	Hyperbil	Control	Hyperbil	Control
GFR (ml/1 73 m² B S /min)	36 0±7 3	53 2±11 1	31 1± 6 3	38 3± 8 1
Gestational age (weeks)	39 6± 1 1	40 8± 0 8	35 0± 0 6	34 4± 1 8
Post natal age (hrs)	116 2±23 3	103 8±11 7	124 2±35 5	132 4±24 7
Hematocrit (%)	49 1± 4 9	48 0± 3 8	57 7± 5 5	41 2± 6 7
n	9	5	6	5

Values are means±S D

ACKNOWLEDGEMENT

This work has been supported by grants from Sällskapet Barnavård Karolinska Institutets fonder and grant 79-19X-03644-08B from the Swedish Medical Research Council

REFERENCES

1 Aylward T D Schowengerdt C G & Bove K Experimental hyperbilirubinemia Effect on glomerular filtration *J Surg Res 15* 1 1973

2 Broberger U Determination of glomerular filtration rate in the newborn *Acta Paediatr Scand 62* 625 1973

3 Broberger U & Aperia A Renal function in idiopathic respiratory distress syndrome *Acta Paediatr Scand 67* 313 1978

4 Calcagno P L & Rubin M I Renal extraction of paraaminohippurate in infants and children *J Clin Invest 42* 1632 1963

5 Dubowitz L M S Dubowitz V & Goldberg C Clinical assessment of gestational age in the newborn infant *J Pediat 77* 1 1970

6 Gekle D & Kuhner U Untersuchungen uber den nephrotoxischen Einfluss des Bilirubins auf die Urinenzymausscheidung Neugeborener *Monatschr Kinderheilk 120* 304 1972

7 Guignard J P Torrado A Mazouni S M & Gautier E Renal function in respiratory distress syndrome *J Pediatr 88* 845 1976

8 Hilger H H Klumper J D & Ullrich K J Wasserruckresorption und Ionentransport durch die Sammelrohrzellen der Saugetierniere *Pfluegers Arch 267* 218 1958

9 Klaus M H & Fanaroff A A *Care of the High risk Neonate* W B Saunders Philadelphia Pa 1973 pp 332 & 337

10 Mia A S Gronwall R R McGavin M D & Cornelius C E Renal function defect in mutant South down sheep with congenital hyperbilirubinemia *Proc Soc Exp Biol Med 137* 1237 1971

11 Nosslin B The direct diazo-reaction of bile pigment in serum *Scand J Clin Lab Invest 12* suppl 49 p 1 1960

12 Odell G B Natschka J C & Storey G N B Bilirubin nephropaty in the Gunn strain of rat *Am J Physiol 212* 931 1967

13 Oh W & Karecki H Phototherapy and insensible water loss in the newborn infants *Am J Dis Child 124* 230 1972

14 Powierza Kaczyńska C Renal function in patients with viral hepatitis and obstructive jaundice *Pol Med J 11* 83 1972

15 Sapirstein L A Vidth D G Mandel M J & Hanusek G Volumes of distribution and clearance of intravenously injected creatinine in the dog *Am J Physiol 181* 330 1955

16 Sitprija V Renal function in jaundice *J Med Assoc Thai 53* 759 1970

17 Smith H W Finkelstein N Alimenosa L Crawford B & Graber M The renal clearances of substituted hippuric acid derivatives and other aromatic acids in dog and man *J Clin Invest 24* 388 1945

18 Zetterstrom R & Ernster L Bilirubin and uncoupler of oxidative phosphorylation in isolated mitochondria *Nature 178* 1335 1955

Submitted April 6 1978
Accepted June 25 1978

(U B) Department of Paediatrics
Karolinska Sjukhuset
Fack
S 104 01 Stockholm 60
Sweden

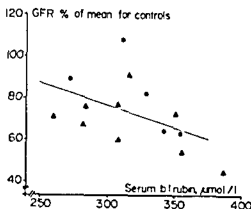


Fig 1 Relation between GFR and serum bilirubin level GFR is expressed as % of the mean for thee respective control group Preterm infants ● Full term infants ▲

significantly higher in infants with jaundice 4 4, than in controls 2 1 mmol/l 73 m² B S /h (0 05>p>0 025) The same was found in 6 pre term infants with hyperbilirubinemia and 5 controls, i e 3 2 and 2 0 mmol/l 73 m² B S /h respectively (0 025>p>0 0125)

DISCUSSION

This study shows that the newborn infant with unconjugated hyperbilirubinemia has a reduced glomerular filtration rate Phototherapy increases stool and insensible water losses (13) and may therefore influence the GFR since dehydration will result in a reduced GFR In this study the hematocrit of the hyperbilirubinemic infants was within the normal range with regard to the postnatal age (9) The amount of fluid given should thus have compensated for the increased water loss The reduction of GFR in the jaundiced infants could therefore be attributed to the hyperbilirubinemia as such Thus, when the serum bilirubin exceeds 325 µmol/l the glomerular filtration rate is reduced to 65% of the expected normal value In contrast to the GFR the PAH clearance was not found to be reduced in jaundiced infants It has been proposed that the development of the PAH transport is delayed due to a decrease either in tubular mass or tubular cell function (4) The PAH clearance should in such a case underestimate the renal plasma flow Provided that there is no difference in the PAH transport between infants with hyperbilirubinemia and control infants hyperbilirubinemia should not affect the renal plasma flow It is unlikely that infants with hyperbilirubinemia have an enhanced transport of PAH If they have a decreased transport of PAH as compared with the control infants the actual renal plasma flow would be higher than in the controls In adults as well as in animals with hyperbilirubinemia of mixed conjugated and unconjugated type and in animals with unconjugated hyperbilirubinemia a significant decrease in the glomerular filtration rate occurs (10 14 16) This has been attributed to a reduction in renal plasma flow as well as to a direct effect of bilirubin on glomerular function The present results are more compatible with the latter explanation

Signs of renal tubular impairment have previously been reported in unconjugated hyperbilirubinemia These signs include enzymuria decreased concentration capacity and sodium wasting (6 12) In the present study both term and pre term infants with hyperbilirubinemia showed a higher sodium excretion after oral sodium loading than did their respective controls Bilirubin is an uncoupler of oxidative phosphorylation in mitochondria (18) The enzymuria originating in the proximal tubular cells of newborn infants with unconjugated hyperbilirubinemia has been attributed to this effect (6) A similar mechanism may cause a decreased sodium transport and thus decrease the sodium reabsorption

The reduced excretory function that has been shown in this study should have clinical implications for the pharmacological treatment of the hyperbilirubinemic newborn This applies especially to the administration of aminoglucosides where a nephro as well as a neurotoxic effect of the drug may be expected The possibility of a negative sodium balance must also be considered during protracted hyperbilirubinemia in the newborn infant

ACKNOWLEDGEMENT

This work has been supported by grants from Sallskapet Barnavård Karolinska Institutets fonder and grant B79-19X-03644-08B from the Swedish Medical Research Council

REFERENCES

1 Aylward T D Schowengerdt C G & Bove K Experimental hyperbilirubinemia Effect on glomerular filtration *J Surg Res 15* 1 1973

Broberger U Determination of glomerular filtration rate in the newborn *Acta Paediatr Scand 62* 625 1973

3 Broberger U & Aperia A Renal function in idiopathic respiratory distress syndrome *Acta Paediatr Scand 67* 313 1978

4 Calcagno P L & Rubin M I Renal extraction of paraaminohippurate in infants and children *J Clin Invest 42* 1632 1963

5 Dubowitz L M S Dubowitz V & Goldberg C Clinical assessment of gestational age in the newborn infant *J Pediatr 77* 1 1970

6 Gekle D & Kuhner U Untersuchungen uber den nephrotoxischen Einfluss des Bilirubins auf die Urinenzymausscheidung Neugeborener *Monatschr Kinderheilk 120* 304 1972

7 Guignard J P Torrado A Mazouni S M & Gautier E Renal function in respiratory distress syndrome *J Ped atr 88* 845 1976

8 Hilger H H Klumper J D & Ullrich K J Wasserruckresorption und Ionentransport durch die Sammelrohrzellen der Saugetierniere *Pfluegers Arch 267* 218 1958

9 Klaus M H & Fanaroff A A *Care of the High risk Neonate* W B Saunders Philadelphia Pa 1973 pp 332 & 337

10 Mia A S Gronwall R R McGavin M D & Cornelius C E Renal function defect in mutant Southdown sheep with congenital hyperbilirubinemia *Proc Soc Exp Biol Med 137* 1237 1971

11 Nosslin B The direct diazo-reaction of bile pigment in serum *Scand J Clin Lab Invest 12* suppl 49 p 1 1960

12 Odell G B Natschka J C & Storey G N B Bilirubin nephropaty in the Gunn strain of rat *Am J Physiol 212* 931 1967

13 Oh W & Karecki H Phototherapy and insensible water loss in the newborn infants *Am J Dis Child* 124 230 1972

14 Powierza Kaczyńska C Renal function in patients with viral hepatitis and obstructive jaundice *Pol Med J 11* 83 1972

15 Sapirstein L A Vidth D G Mandel M J & Hanusek G Volumes of distribution and clearance of intravenously injected creatinine in the dog *Am J Physiol 181* 330 1955

16 Sitprija V Renal function in jaundice *J Med Assoc Thai 53* 759 1970

17 Smith H W Finkelstein N Aliminosa L Crawford B & Graber M The renal clearances of substituted hippuric acid derivatives and other aromatic acids in dog and man *J Clin Invest 24* 388 1945

18 Zetterstrom R & Ernster L Bilirubin and uncoupler of oxidative phosphorylation in isolated mitochondria *Nature 178* 1335 1955

Submitted April 6 1978
Accepted June 25 1978

(U B) Department of Paediatrics
Karolinska Sjukhuset
Fack
S 10401 Stockholm 60
Sweden

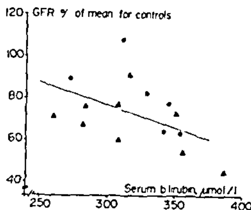


Fig 1 Relation between GFR and serum bilirubin level GFR is expressed as % of the mean for thee respective control group Preterm infants ● Full term infants ▲

significantly higher in infants with jaundice, 4 4 than in controls 2 1 mmol/1 73 m² B S /h (0 05>p>0 025) The same was found in 6 pre term infants with hyperbilirubinemia and 5 controls i e 3 2 and 2 0 mmol/1 73 m² B S /h respectively (0 025>p>0 0125)

DISCUSSION

This study shows that the newborn infant with unconjugated hyperbilirubinemia has a re duced glomerular filtration rate Phototherapy increases stool and insensible water losses (13) and may therefore influence the GFR since de hydration will result in a reduced GFR In this study the hematocrit of the hyperbilirubinemic infants was within the normal range with re gard to the postnatal age (9) The amount of fluid given should thus have compensated for the increased water loss The reduction of GFR in the jaundiced infants could therefore be attributed to the hyperbilirubinemia as such Thus when the serum bilirubin exceeds 325 µmol/l the glomerular filtration rate is re duced to 65% of the expected normal value In contrast to the GFR the PAH clearance was not found to be reduced in jaundiced infants It has been proposed that the development of the PAH transport is delayed due to a decrease either in tubular mass or tubular cell function (4) The PAH clearance should in such a case underestimate the renal plasma flow Provided that there is no difference in the PAH transport between infants with hyperbilirubinemia and control infants hyperbilirubinemia should not affect the renal plasma flow It is unlikely that infants with hyperbilirubinemia have an enhanced transport of PAH If they have a decreased transport of PAH as compared with the control infants the actual renal plasma flow would be higher than in the controls In adults as well as in animals with hyperbiliru binemia of mixed conjugated and unconjugat ed type and in animals with unconjugated hy perbilirubinemia a significant decrease in the glomerular filtration rate occurs (10 14 16) This has been attributed to a reduction in renal plasma flow as well as to a direct effect of bili rubin on glomerular function The present re sults are more compatible with the latter ex planation

Signs of renal tubular impairment have pre viously been reported in unconjugated hyper bilirubinemia These signs include enzymuria decreased concentration capacity and sodium wasting (6 12) In the present study both term and pre term infants with hyperbilirubinemia showed a higher sodium excretion after oral sodium loading than did their respective con trols Bilirubin is an uncoupler of oxidative phosphorylation in mitochondria (18) The enzymuria originating in the proximal tubular cells of newborn infants with unconjugated hyperbilirubinemia has been attributed to this effect (6) A similar mechanism may cause a decreased sodium transport and thus decrease the sodium reabsorption

The reduced excretory function that has been shown in this study should have clinical implications for the pharmacological treatment of the hyperbilirubinemic newborn This applies especially to the administration of aminoglucosides where a nephro as well as a neurotoxic effect of the drug may be expected The possibility of a negative sodium balance must also be considered during protracted hyperbilirubinemia in the newborn infant

Acta Pædiatr Scand 68 81–84 1979

MONONUCLEAR CELL MIGRATION INHIBITION IN CHILDREN WITH NEPHROTIC SYNDROME

K KUCHARSKA D KOWALCZYK K SANCEWICZ PACH and M ZEMBALA

From the Institute of Paediatrics M dical Academy I rakow Poland

ABSTRACT Kucharska K Kowalczyk D Sancewicz Pach K and Zembala M (Institute of Paediatrics Medical Academy Kraków Poland) Mononuclear cell migration inhibition in children with nephrotic syndrome Acta Paediatr Scand 68 81 1979 —The relative number of T/B lymphocytes and the response of peripheral blood mononuclear cells to PHA in the migration inhibition test was studied in a group of patients with idiopathic nephrotic syndrome It has been found that the level of T lymphocytes and the response to PHA was decreased in patients in particular those with frequent relapses The possible implications of these finding for pathogenesis of idiopathic nephrotic syndrome is briefly discussed

KEY WORDS Nephrotic syndrome T cell function mononuclear cell migration inhibition

The etiology of the idiopathic nephrotic syndrome with minimal changes in glomeruli is unknown The response to steroid and cyclophosphamide therapy is considered evidence of an immunological background (3 6) but against this are the normal blood levels of complement components including beta, globulin and the absence of glomerular deposits of immunoglobulins and C3 Also to be considered is the possibility of dysfunction of cell mediated immunity (7) It has been reported that children with minimal changes idiopathic nephrotic syndrome do not respond to sensitization with dinitrochlorobenzene DNCB (8) In addition some of the affected children have a past history of allergic conditions (2)

Shalhoub (3) suggested that the T cells of nephrotic patients produce a lymphokine which is toxic for the glomerular basement membrane and increases its permeability to proteins leading to the development of nephrotic syndrome

The present studies were designed to assess two aspects of cell mediated immunity in children with idiopathic nephrotic syndrome the relative number of T lymphocytes and the response of peripheral blood mononuclear (MN) cells to PHA in the migration inhibition test As we have shown previously this test is a measure of T lymphocytes response to lymphokines produced by MN cells (10)

MATERIAL AND METHODS

Patients

15 children with idiopathic nephrotic syndrome aged from 19/12 to 11 years (mean 2 6/12) were studied Six of these children were studied at the onset of the disease before prednisone therapy was started The remaining patients were observed during second or subsequent relapses Blood was taken at least 6 weeks after cessation of therapy The standard therapy consisted of administration of prednisone 1–? mg/kg/24h for a period of 3–8 weeks

The clinical status of all the children was similar during the period of study The patients were divided into ? groups —8 children with good response to prednisone therapy and 11—7 children with poor response to prednisone therapy relapsing over 4 times a year i e frequent relapsers In three children from the second group renal biopsy was performed during the 6th or ? relapses Minimal changes in the glomeruli were present Renal function tests were normal in all patients Six healthy age matched children served as controls (Table 1)

Isolation of peripheral blood mononuclear cells and their surface markes

Mononuclear cells (MN) were isolated from the EDTA venous blood by standard Sodium Metrizoate Ficoll technique (5) T lymphocytes were scored by their ability to from rosettes with sheep erythrocytes E RCF (9) with minor modifications (14)

Acta Pædiatr Scand 68 81–84 1979

MONONUCLEAR CELL MIGRATION INHIBITION IN CHILDREN WITH NEPHROTIC SYNDROME

K KUCHARSKA D KOWALCZYK K SANCEWICZ PACH and M ZEMBALA

From the Institute of Paediatrics M dical Academy Krakow Poland

ABSTRACT Kucharska K Kowalczyk D Sancewicz Pach K and Zembala M (Institute of Paediatrics Medical Academy Kraków Poland) Mononuclear cell migration inhibition in children with nephrotic syndrome Acta Paediatr Scand 68 81 1979.—The relative number of T/B lymphocytes and the response of peripheral blood mononuclear cells to PHA in the migration inhibition test was studied in a group of patients with idiopathic nephrotic syndrome It has been found that the level of T lymphocytes and the response to PHA was decreased in patients in particular those with frequent relapses The possible implications of these finding for pathogenesis of idiopathic nephrotic syndrome is briefly discussed

KEY WORDS Nephrotic syndrome T cell function mononuclear cell migration inhibition

The etiology of the idiopathic nephrotic syndrome with minimal changes in glomeruli is unknown The response to steroid and cyclophosphamide therapy is considered evidence of an immunological background (3 6) but against this are the normal blood levels of complement components including beta$_{1c}$globulin and the absence of glomerular deposits of immunoglobulins and C3 Also to be considered is the possibility of dysfunction of cell mediated immunity (7) It has been reported that children with minimal changes idiopathic nephrotic syndrome do not respond to sensitization with dinitrochlorobenzene DNCB (8) In addition some of the affected children have a past history of allergic conditions (2)

Shalhoub (3) suggested that the T cells of nephrotic patients produce a lymphokine which is toxic for the glomerular basement membrane and increases its permeability to proteins leading to the development of nephrotic syndrome

The present studies were designed to assess two aspects of cell mediated immunity in children with idiopathic nephrotic syndrome the relative number of T lymphocytes and the response of peripheral blood mononuclear (MN) cells to PHA in the migration inhibition test As we have shown previously this test is a measure of T lymphocytes response to lymphokines produced by MN cells (10)

MATERIAL AND METHODS

Patients

15 children with idiopathic nephrotic syndrome aged from 19/12 to 11 years (mean 2 6/12) were studied Six of these children were studied at the onset of the disease before prednisone therapy was started The remaining patients were observed during second or subsequent relapses Blood was taken at least 6 weeks after cessation of therapy The standard therapy consisted of administration of prednisone 1–2 mg/kg/24h for a period of 3–8 weeks

The clinical status of all the children was similar during the period of study The patients were divided into 2 groups —8 children with good response to prednisone therapy and II—7 children with poor response to prednisone therapy relapsing over 4 times a year i e frequent relapsers In three children from the second group renal biopsy was performed during the 6th or 7th relapses Minimal changes in the glomeruli were present Renal function tests were normal in all patients Six healthy age matched children served as controls (Table I)

Isolation of peripheral blood mononuclear cells and their surface markes

Mononuclear cells (MN) were isolated from the EDTA venous blood by stardard Sodium Metrizoate Ficoll technique (5) T lymphocytes were scored by their ability to from rosettes with sheep erythrocytes E RCF (9) with minor modifications (14)

6 787871

Table 1 *Clinical data of children with the nephrotic syndrome*

No	Ini tials	Age	Sex	Age at the beginning of INS	Re lapse	Laboratory findings: Urinary protein (g/day)	Serum albumin (g/l)	Serum cholesterol (mmol/l)	Renal biopsy
1	J I	2	F	2	I	4 5	12 3	10 0	
2	P M	19/12	F	18/12	I	33 7	13 2	16 1	
3	Cz M	2	F	2	I	33 0	13 6	8 1	
4	D K	3	M	3	I	4 2	13 3	10 0	
5	E A	2	M	2	I	16 3	14 1	9 5	
6	J G	6	M	6	I	3 0	16 2	10 7	
7	C B	6	F	3	II	3 2	16 3	9 3	
8	K R	3 5	M	14/12	III	3 8	10 3	17 2	
9	M R	5	F	2	IV	3 9	17 8	10 0	
10	N R	5	M	3	IV	12 4	14 2	9 7	
11	W J	8	M	6	IV	2 0	17 6	8 0	
12	T R	3 5	M	14/12	VI	2 5	18 0	8 0	
13	B K	3	F	17/12	VI	33 7	17 1	8 4	Minimal changes
14	K K	11 5	F	2	VII	28 0	24 0	6 0	Minimal changes
15	Z M	8	F	18/12	VII	2 5	10 4	8 0	Minimal changes

B lymphocytes bearing receptors for C3 were assessed by their ability to form rosettes with erythrocytes coated with IgM antisheep red cell antibody complement—EAC rosettes (4)

Migration inhibition test

Capillary tubes were filled with MN suspensions contain ing 3×10^7 cells/ml After centrifugation the capillaries were cut at the fluid cell interface and placed into migra tion chambers plates (Sterilin) The tests were run in triplicates Eagle s minimal essential medium supple mented with 10% foetal calf serum and antibiotics was used

Purified phytohaemagglutinin (Wellcome Beckenham Kent England) at the concentration 1 μm/ml was used In the control chambers PHA was omitted For the details see (10) The migration index (M I) was calculated using the standard formula (2)

$$M\ I = \frac{\text{area of migration in the presence of PHA}}{\text{area of migration without PHA}}$$

Statistical evaluation was done according to Student s *t* test

RESULTS

Table 2 shows that the relative values of T lymphocytes were decreased in the patients as compared with the controls This was more marked in the group with poor response to treatment The level of B cells was increased in both groups of patients These differences were however statistically non significant ($p>0\ 05$)

Table 2 also shows that migration inhibition indices were higher i e the response to PHA was decreased in the patient group as com pared to with the normal subjects The re

Table 2 *The relative number of T and B lymphocytes and migration indices in children with INS*

Group	No of pa tients	Relative num ber of T lymphocyte ($x\pm$S E)	Relative num ber of B lymphocyte ($x\pm$S E)	Migration index ($x\pm$S E)
Patients with ne phrotic syndrom (total)	15	65 4 ±4 54	16 97±1 38	0 63±0 05
Group I	8	71 8 ±3 33	17 25±1 09	0 58±0 04
Group II	7	64 ±6 78	16 35±2 44	0 73 ±0 08
Control	6	75 66±1 47	13 25±2 34	0 47±0 04

$p<0\ 05$

spon e of group II patients was decreased more than that of group I The M I was re markably high in 4 children 0 77 0 96 0 96 and 0 99 indicating no response to PHA After prednisone therapy the values fell and reactiv ity to PHA was regained

It was concluded that the response of pa tients cells to PHA was depressed This dif ference was statistically significant only for patients from group II

DISCUSSION

Our results seem to indicate that cell mediated immunity is altered in the idiopathic nephrotic syndrome Patients in the early stage of the disease did not show any significant changes while children experiencing many relapses showed a decreased number of T lymphocytes and poor production of lymphokines as judged from the results of migration inhibition De creased numbers of T lymphocytes in children with nephrotic syndrome were also found by Baliah et al (1)

The finding of a depressed response to PHA in the migration inhibition test is in keeping with observations that during the acute phase patients with idiopathic nephrotic syndrome have markedly decreased lymphocyte trans formation to PHA (11) It is also interesting that delayed hypersensitivity skin reactions are depressed in nephrotic patients (8) Schul te Wisserman et al (12) postulated that in pa tients with idiopathic nephrotic syndrome cir culating T suppressor cells are responsible for the depressed blast transformation to PHA These cells may act via soluble factors These suppressor cells may also be responsible for the depressed response in the migration inhibi tion test inhibiting a lymphokine production by patients lymphocytes

Our preliminary observations indicate that in patients whose cells did not respond to PHA in the migration inhibition test this ability was regained after therapy and clinical improve ment Similar results were obtained by Schul te Wisserman et al (12) in the lymphocyte transformation test They postulated that the effect of therapy is best explained by preferen tial action of steroids on T suppressor cells

The role of depressed cell mediated immu nity in the pathogenesis of INS is not clear The disease may represent a T cell disorder Our results support the notion that suppressor T cells and soluble factors produced by them may on one hand inhibit lymphocyte reactiv ity and on the other damage the glomerular basement membrane (12) This is in keeping with Shalhoub s (13) hypothesis

At the practical level our results indicate that these simple tests of cellular reactivity may be clinically useful in monitoring the ef fect of therapy Further and more detailed studies on a larger group of patients are needed to show whether the immunological mechanisms proposed are involved in the pathogenesis of the disease

ACKNOWLEDGEMENTS

This work received financial support from Polish Academy of Sciences (grants no 437/VI and 10 5) The skillful technical assistance of Mrs Irena Ruggiero is gratefully acknowledged

REFERENCES

1 Baliah T Neter E Li P K Cohen E & Park B H Immunologic studies in minimal lesion ne phrotic syndrome in children Abstracts *Fourth International Symposium of Paediatric Nephrology* Helsinki 1977 81

2 Balow J E Hurley D L & Fanci A S Im munosupressive effects of glucocorticosteroids dif ferential effects of acute VS chronic administration on cell mediated immunity *J immunol 114* 1072 1975

3 Barrat T M & Soothill J F Controlled trial of cyclophosphamide in steroid—sensitive relapsing ne phrotic syndrome of childhood *Lancet II* 479 1970

4 Bianco C Patrick R & Nussenzweig V A pop ulation of lymphocytes bearing a membrane receptor for antigen–antibody complement comp ex I Sepera tion and characterization *J Exp Med 132* 70 1970

5 Boyum A Separation of leukocytes from blood and bone marrow *Scand J Cl n Lab Invest 21* Suppl 97 51 1968

6 Cameron J S Bright s disease today The patho genesis and treatment of glomerulonephritis *Br Med J IV* 87 1972

7 Eyres K Mallick N P & Taylor G Leukocyte migration inhibition in minimal change nephropathy *Lancet II* 198 1976

8 Halikowski B Korczowski R & Zajączkowski J Odczyn skórny na dwunitrochlorobenzen (DNCB) u nosicieli antygenu Australia *Pediatr Pol 47* 1071 1972
9 Jondal M Holm G & Wigzell H Surface markers on human T and B lymphocytes forming non immune rosettes with sheep red blood cells *J Exp Med 136* 207 1972
10 Kowalczyk D & Zembala M Migration inhibition of T lymphocytes from human peripheral blood by specific antigens and lymphokines *Clin Exp Immunol* 1978 (in press)
11 Moorthy A V Zimmerman S W & Burkholder P M Inhibition of lymphocyte blastogenesis by plasma of patients with minimalchange nephrotic syndrome *Lancet I* 1160 1976
12 Schulte Wissermann H Lemmel E M Reitz M Beck J & Straub E Nephrotic syndrome of childhood and disorder of T cell function *Eur J Pediatr 124* 121 1977
13 Shalhoub R J Pathogenesis of lipoid nephrosis A disorder of T cell function *Lancet II* 556 1974
14 Zembala M Mytar B Popiela T & Asherson G L Depressed in vitro peripheral blood lymphocyte response to mitogens in cancer patients the role of suppressor cells *Int J Cancer 19* 605 1977

Submitted March 13 1978
Accepted May 19 1978

(K K) Institute of Paediatrics
Medical Academy
Wielicka 265
30-663 Kraków
Poland

Acta Pædiatr Scand 68 85–92 1979

PREDICTION OF ADULT HEIGHT WITH VARIOUS METHODS IN FINNISH CHILDREN

HANNA LIISA LENKO

From the Children s Hospital University of Helsinki Finland

ABSTRACT Lenko H L (Children s Hospital University of Helsinki Finland) Prediction of adult height with various methods in Finnish children Acta Paediatr Scand 68 85 1979 —Successive height predictions were made by several methods for a group of healthy Finnish children (30 boys and 30 girls) examined annually at ages of 7 to 17 years (1st series) and for 7 boys aged 14 to 19 years with familial delayed growth and puberty (2nd series) The methods used were those of Bayley & Pinneau (BP) Walker (W) Tanner et al (T) and RWT and two simple principles the relative height method (RH) which assumes constancy of height S D S throughout growth and the index of potential height (IPH) method which assumes constancy of height S D S for bone age (BA) The predictions with RH W and IPH were inaccurate BP T and RWT were for the 1st series as accurate as for the basic series of the respective methods and none was superior to the others The BA of average Finns was delayed as compared with the standards of Greulich Pyle Atlas When corrections were made for this delay the IPH method gave predictions comparable in accuracy to BP T or RWT In the 2nd series prediction was more accurate with the corrected IPH BP and RWT methods than with those using BA according to TW 2 RUS

KEY WORDS Growth height prediction bone age

Several new methods have recently been developed for the prediction of adult height (9 12 14) Present height weight bone age mid parent height growth velocity and pubertal stage are variably included in the prediction equations and so for the same child each method gives a somewhat different prediction Applying these methods to series of children whose final heights are known should allow a comparative evaluation of the methods but no such study has previously been published This is a report of such a comparison of the different methods in a series of healthy Finnish children

MATERIALS AND METHODS

S bjects

Firstly pred ctions of adult height were made from data obtained yearly at ages 8–17 years for series of 30 boys and 30 girls participants in a Finnish longitudinal growth study (5) whose heights at age 18 years were known All were of Finnish ancestry born in the Helsinki area and free from chronic disease They had been seen yearly within one month of their birthdays For calculations the chronological age (CA) was rounded to whole years Most of the subjects had missed one or more visits the actual number of subjects of each sex seen at each age varied be

List of abbreviations

CA	Chronological age
G BA	Greulich Pyle bone age
T BA	TW 2 RUS bone age
S D S	Standard deviation score

Methods for height prediction

RH	Relative height (assumes that the present and the final height S D S are equal)
IPH	Index of potential height (assumes that present and the final height S D S for bone age are equal)
IPH-G	IPH calculated at G BA
IPH-Gc	IPH calculated at corrected G BA
IPH T	IPH calculated at T BA
IPH Tc	IPH calculated at corrected T BA
W 1	Walker method with height and CA
W 2	Walker method with height CA and growth rate
BP	Bayley Pinneau method
T 1	Tanner et al method with CA dependent coefficients
T 2	Tanner et al method with T BA dependent coefficients
RWT	Roche Wainer and Thissen method

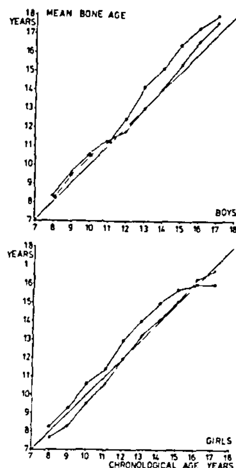


Fig 1 Mean Greulich Pyle (O) and TW 2 RUS (●) bone ages at different chronological ages in the first series of subjects Identity is indicated by the diagonal

tween 16 and 28 (mean 23 5) Heights had been meas ured to the nearest 0 1 cm with the Martin anthropometer and weights to the nearest 0 1 kg The measurements were always made by one of three observers The heights at 18 years were taken as the adult heights although some growth takes place after this age Of the 18 boys and 20 girls whose later heights were known 9 and 4 had grown >0 5 and 2 and 2 >1 cm respectively The mean adult heights were 177 5 cm±5 9 cm (S D) and 164 1 cm±5 1 cm while the means reported for young Finnish males and females are 177 2 cm ±6 0 cm and 165 1 cm ±5 0 cm respectively (2)

Secondly similar predictions were made for a series of 7 healthy boys with familial delayed growth and puberty They had been followed at our endocrine clinic and their adult heights were known Full data for their total of 20 visits between the ages of 14 and 19 years were available

Bone age

Bone age (BA) was determined from roentgenograms of hand and wrist taken at each visit Both the Greulich Pyle Atlas (G BA) (4) and the TW2RUS method (T BA) (13) were used For the G BA the developmental stage of the epiphyses was compared with the standards the car pal round bones being disregarded (1) and G BA was determined by interpolation to the nearest 0 25 years

All BAs were determined independently by myself and another pediatrician without knowledge of the identity or CA of the child The mean of the two values was used in calculations When they differed by >0 5 years (this occurred in 103 cases with G BA and 130 cases with T BA of the total of 469) we made repeat determinations with out knowledge of the previous values The mean of the second values was accepted even if the difference was again >0 5 years (24 and 31 cases respectively) For T BA no systematic difference was present between the two sets of readings For G BA one of us tended to ob tain higher values than the other for girls the mean dif ference was 0 1 years which is statistically significant at the level $p<0\ 001$

Height prediction

Relative height method (RH) This assumes that the final height standard deviation score (S D S) (the devia tion + or − in S D units from the mean for age and sex) will be the same as the present S D S The S D s were obtained from current standard growth curves for Finnish children based on a cross sectional study (?)

Index of potential height methods (IPH) (7) These assume that the height S D S remains unchanged when calculated for BA (rather than for CA as in the RH meth od) IPH was calculated for both G BA (IPH G) and T BA (IPH T)

Walker methods (W) (14) W 1 employs height and CA and W 2 in addition the growth rate over the preceding year W3 was not used because the necessary CA at peak height velocity was not always known

Bayley and Pinneau method (BP) (3) This well known method employs tables giving the percentages of final height that are acquired at each G BA for average early and late maturers

Tanner methods (T) (12) These equations contain coef ficients for height CA and T BA The coefficients are specific for CA (method T 1) or for T BA (T 2) The equa tions that include midparent height were not used

RWT method (9) This employs recumbent length weight midparent height and G BA The recumbent length was obtained by adding 1 25 cm to the observed standing height (9) The tables are to be used only until half of the bones of the hand wrist are mature (9) Our different usage of the Greulich Pyle standards did not allow such discrimination and the tables were used up to CA 14 years for girls and 16 years for boys For the sec ond series the coefficients for 16 years were used even at older age

RESULTS

Bone age

The two methods gave different BAs In girls and in pubertal boys G BA was almost always lower than T BA In prepubertal boys individ ual differences were larger but there was no systematic trend *In prepubertal girls G BA* tended to be lower than CA (Fig 1), but the

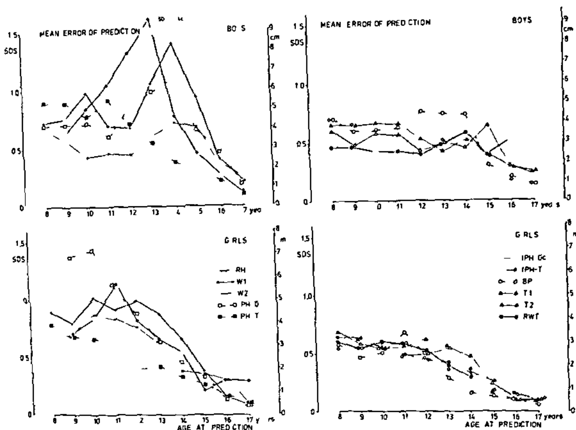


Fig 2 Mean absolute errors of yearly predictions by the different methods in the first series of subjects For abbreviations see footnote on page 1

difference was not statistically significant In boys the mean G BA was higher than CA except at 12–14 years and this difference was statistically significant at 10 and 17 years The mean T BAs were almost consistently higher than CA and this difference was statistically significant at 10 and 12–15 years in girls and at 13–16 years in boys

Accuracy of prediction

Fig 2 shows the mean of the absolute values of differences between the adult heights predicted and attained Those methods of prediction that are based on CA and disregard earliness or lateness of maturation i e methods RH and W were inaccurate with the exception of W1 in boys The use of BA instead of CA (IPH G and IPH T) improved the prediction only in the case of IPH T in girls The more elaborate methods BP T and RWT were significantly better No clear differences were evident between the accuracies of these methods either individually or as based on G BA or T BA The systematic errors were largest with RH W and IPH In general overprediction was more common for boys than for girls Methods using T BA (IPH T T1 and T2) gave overpredictions before puberty and underpredictions in puberty [1]

Consistency of predictions

For early evaluation of the effect of an intervention such as androgen treatment on final height consecutive predictions have to be

[1] Detailed table of the statistics of systematic errors for each method age and sex can be obtained at request from the author

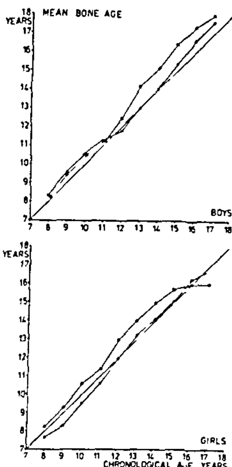


Fig 1 Mean Greulich Pyle (O) and TW2 RUS (●) bone ages at different chronological ages in the first series of subjects Identity is indicated by the diagonal

tween 16 and 28 (mean 23 5) Heights had been measured to the nearest 0 1 cm with the Martin anthropometer and weights to the nearest 0 1 kg The measurements were always made by one of three observers The heights at 18 years were taken as the adult heights although some growth takes place after this age Of the 18 boys and 20 girls whose later heights were known 9 and 4 had grown >0 5 and 2 and 2 >1 cm respectively The mean adult heights were 177 5 cm±5 9 cm (S D) and 164 1 cm±5 1 cm while the means reported for young Finnish males and females are 177 2 cm ±6 0 cm and 165 1 cm ±5 0 cm respectively (2)

Secondly similar predictions were made for a series of 7 healthy boys with familial delayed growth and puberty They had been followed at our endocrine clinic and their adult heights were known Full data for their total of 20 visits between the ages of 14 and 19 years were available

Bone age

Bone age (BA) was determined from roentgenograms of hand and wrist taken at each visit Both the Greulich Pyle Atlas (G BA) (4) and the TW2RUS method (T BA) (13) were used For the G BA the developmental stage of the epiphyses was compared with the standards the carpal round bones being disregarded (1) and G BA was determined by interpolation to the nearest 0 25 years

All BAs were determined independently by myself an another pediatrician without knowledge of the identity o CA of the child The mean of the two values was use in calculations When they differed by >0 5 years (thi occurred in 103 cases with G BA and 130 cases with T B of the total of 469) we made repeat determinations with out knowledge of the previous values The mean of the second values was accepted even if the difference wa again >0 5 years (24 and 31 cases respectively) Fo T BA no systematic difference was present between th two sets of readings For G BA one of us tended to obtain higher values than the other for girls the mean di ference was 0 1 years which is statistically significant a the level $p<0\ 001$

Height prediction

Relative height method (RH) This assumes that th final height standard deviation score (S D S) (the deviation + or − in S D units from the mean for age an sex) will be the same as the present S D S The S D were obtained from current standard growth curves fo Finnish children based on a cross sectional study (7)

Index of potential height methods (IPH) (7) Thes assume that the height S D S remains unchanged whe calculated for BA (rather than for CA as in the RH method) IPH was calculated for both G BA (IPH G) and T B (IPH T)

Walker methods (W) (14) W 1 employs height and CA and W 2 in addition the growth rate over the preceding year W3 was not used because the necessary CA at peak height velocity was not always known

Bayley and Pinneau method (BP) (3) This well known method employs tables giving the percentages of final height that are acquired at each G BA for average early and late maturers

Tanner methods (T) (12) These equations contain coefficients for height CA and T BA The coefficients are specific for CA (method T 1) or for T BA (T 2) The equations that include midparent height were not used

RWT method (9) This employs recumbent length weight midparent height and G BA The recumbent length was obtained by adding 1 25 cm to the observed standing height (9) The tables are to be used only until half of the bones of the hand wrist are mature (9) Our different usage of the Greulich Pyle standards did not allow such discrimination and the tables were used up to CA 14 years for girls and 16 years for boys For the second series the coefficients for 16 years were used even at older age

RESULTS

Bone age

The two methods gave different BAs In girls and in pubertal boys G BA was almost always lower than T BA In prepubertal boys individual differences were larger but there was no systematic trend In prepubertal girls G BA ter

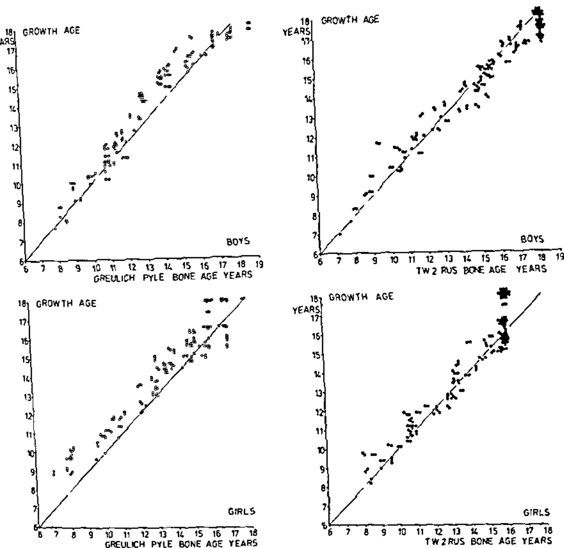


Fig 4 Bone age plotted against corresponding growth age (age at which the actual would equal the final height S D S) in the first series of subjects Identity is indicated by the diagonal

T In the T methods the BA is not given enough weight in situations where the difference between CA and BA is large

DISCUSSION

The present observations are fairly close to those made in the original series of the developers of the various methods The W methods gave quite large errors W 1 gave reasonably good predictions for boys but not for girls and W 2 was poor for both sexes This may be due to differences between the observed populations of New Haven and Finland in timing of growth and in final height Different timing of pubertal growth and the imprecision of the estimates of growth rate (which cumulates the imprecisions of two height measurements) presumably explain the lesser accuracy of W 2 as this includes growth rate With BP the systematic errors were slightly larger in my series than in the basic and validating series of Bayley and Pinneau BP tended to give overprediction especially for the boys The dispersion of the errors was wider in my first series than in Bayley and Pin

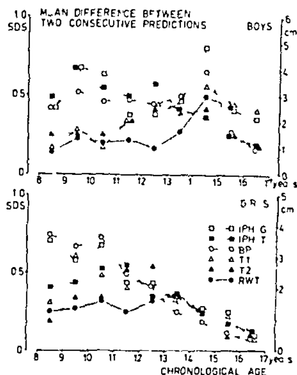


Fig 3 Mean differences between each two consecutive yearly predictions by the different methods in the first series of subjects For abbreviations see footnote on p 1

compared The reliability of such evaluations has to be judged in terms of the consistency of consecutive predictions in the absence of any intervention The mean differences between two consecutive predictions for the first series are shown in Fig 3 for methods BP T RWT, IPH G and IPH T The consistency is relatively poor especially for the methods in which the influence of BA is greatest (BP IPH)

Correction of bone age

RH give overpredictions at all ages The systematic error was largest at ages just before the steepest pubertal part of the Finnish standard growth curves Clearly the pubertal growth spurt occurred earlier in the present series than in the average Finnish children on whom the standards were based The G BAs are not so advanced over CA (Fig 1) Growth age the age at which the child s actual height S D S would have been identical with his or her final S D S was calculated to the nearest 0 1 year for each child at each CA These growth ages were plotted against the corresponding BAs (Fig 4) The G BAs were systematically higher than the corresponding growth ages but the dispersion was smaller than with the T BAs which agreed better with the growth ages The BA growth age pairs were classed separately for boys and girls firstly according to BA and secondly according to growth age Full years were used as class limits The mean growth age was calculated for each BA class and the mean BA for each growth age class All these means were then plotted growth age versus BA A curve smoothed by eye was drawn through these plots (Fig 5) These curves were used for correction of BAs, the growth age being taken for the corrected BA These curves show that the average Finnish children on whom the standards were based were delayed in their skeletal maturation as compared with the Greulich Pyle standards especially at puberty Compared with the T BA standards no such difference was present for boys but girls under 13 years old showed a slight advance

Effect of correction of BA on the accuracy of prediction

The individual BAs were corrected for the systematic error according to Fig 5 With the G BA so corrected IPH had a markedly improved accuracy (see Fig 2 IPH Gc) Correction of T BA did not similarly increase the accuracy of IPH T The IPH methods with the corrected BAs were similar in consistency to BP

Height prediction in boys with delayed puberty

The G BAs of these boys lagged behind CA by 2–5 years and the T BAs by 0 5–5 years Fig 6 shows the mean absolute errors of the first predictions made with different methods for each boy Predictions based on later data did not differ significantly from the first predictions In these boys the G BA seemed to give a more correct measure of maturity than the T BA as the methods IPH Gc BP and RWT were all more accurate than IPH T and

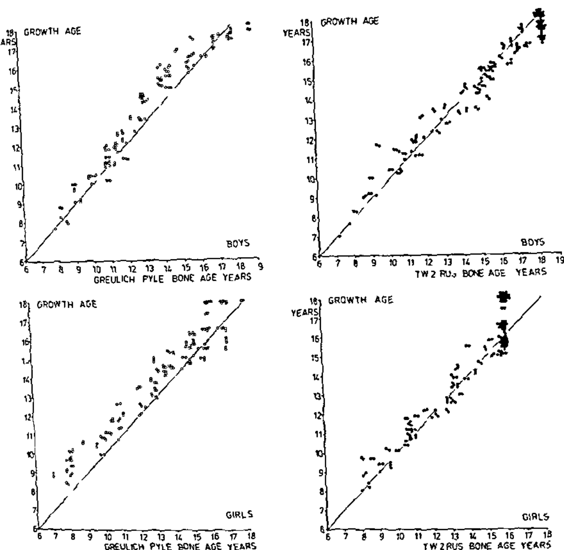


Fig 4 Bone age plotted against corresponding growth age (age at which the actual would equal the final height S D S) in the first series of subjects Identity is indicated by the diagonal

T In the T methods the BA is not given enough weight in situations where the difference between CA and BA is large

DISCUSSION

The present observations are fairly close to those made in the original series of the developers of the various methods The W methods gave quite large errors W1 gave reasonably good predictions for boys but not for girls and W2 was poor for both sexes This may be due to differences between the observed populations of New Haven and Finland in timing of growth and in final height Different timing of pubertal growth and the imprecision of the estimates of growth rate (which cumulates the imprecisions of two height measurements) presumably explain the lesser accuracy of W2 as this includes growth rate With BP the systematic errors were slightly larger in my series than in the basic and validating series of Bayley and Pinneau BP tended to give overprediction especially for the boys The dispersion of the errors was wider in my first series than in Bayley and Pin

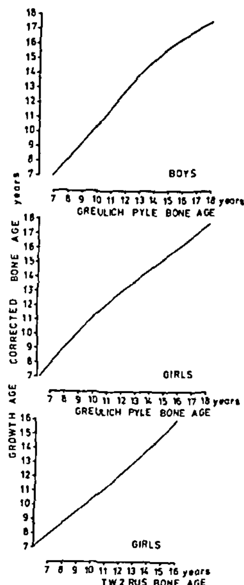


Fig 5 Correction curves for bone age according to the average growth of Finns (see text)

neau s basic sample as expected but it was usually smaller than in their validating sample With the T methods the S D s of the errors were comparable to the residual S D s found by Tanner The girls of 9–12 years in my series were less accurately predicted than the ballet dancers of Tanner but the difference is not statistically significant With RWT the median absolute error and the 90th percentile of absolute error of prediction did not greatly differ in my series as compared with the children in the original Fels growth study In the Finnish sample the predictions showed a larger error when based on data for both boys and girls under 10 years but at later ages were even

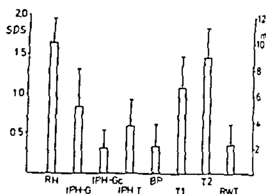


Fig 6 Absolute errors (mean + S D) in height prediction by the different methods in boys with familial delayed growth and puberty (second series of subjects) For abbreviations see footnote on p 1

more accurate than those in the original series Our modified method for determining G BA may explain the slight systematic overprediction for both sexes obtained with RWT Yet with BP using the same BA girls were systematically underpredicted Thus it appears that although different populations differ in the timing of growth the methods which use BA in addition to CA give as good an accuracy for Finnish children as for American or English children

Children are said to have a tendency to follow their own genetic growth channels (6) But this rule applied in our RH method did not lead to good predictions of adult height It was surprising how little these predictions were improved by using BA to allow for differences in timing of growth One source of error is that the Finnish growth standards are cross sectional rather than longitudinal and thus do not present the actual shape of growth curves at puberty (11) Further the average Finn differs in the timing of growth and maturation from the populations on which the BA standards were based and the BAs must be corrected to Finnish growth standards as I have now done

One of the main sources of inaccuracy in prediction of height is the inaccuracy of the BA estimation This estimation is always somewhat subjective With experience the observer reduces the variance of his estimates but between observer differences cannot be

avoided (1) A small difference in BA determination can lead to a great difference in height prediction especially during the pubertal spurt The G BA was somewhat more reproducible than the T BA according to comparison between my two observers readings The T BA places much weight on the maturation of some bones and a one stage difference can cause a year s difference in BA No one knows which criteria of bone maturation are the most significant for prediction of height Neither is it clear whether the hand wrist BA corresponds best to growth as different parts of the skeleton differ in maturation The knee has been suggested to be the most informative for assessing growth potential (10) but height prediction methods based on the knee BA have not been published

The different methods of height prediction have a similar accuracy but require a very variable amount of calculation G BA is easier to determine than T BA The T BA is also subject to calculation errors The IPH methods are rapid and adequate The BA scales should be checked and if necessary corrected for every population For Finnish children T BA needed less correction than G BA Therefore IPH Tc might seem better for Finns than IPH Gc However IPH Tc shows greater variance and a tendency to overprediction before puberty and underprediction during puberty and so may lead to serious misinterpretations of the effects of interventions BP is very simple and gives reasonably accurate predictions The T methods involve more cumbersome calculations especially if the coefficients are extrapolated to the exact age as suggested by the authors In healthy children T 1 and T 2 agreed well RWT seems to be the most accurate but involves the greatest amount of calculations its inaccuracy increased with age and it should be used only up to the BA stated by its authors Both with RWT and with T the predictions may be less than the actual height already achieved

It is not known whether these methods are applicable to children with growth disorders and at the extremes of normal variation Yet it is in these situations that height predictions are most commonly and most urgently needed BP and T 1 have been reported to be accurate in tall girls (8) (15) and T 1 in tall boys (16) but the number of observations was small In our group of boys with delayed puberty appropriate predictions were obtained with IPH BP and RWT but not with T

ACKNOWLEDGEMENT

I am indebted to Dr R L Kantero for allowing me to use for this study the longitudinal data on normal children collected by the Finnish Centre for Study in Child Growth and Development

REFERENCES

1 Acheson R M Fowler G Fry E I Janes M Koski K Urbano P & van der Werff Ten Bosch J J Studies in the reliability of assessing skeletal maturity from X rays Part I Greulich Pyle Atlas *Hum Biol 37* 317 1963
2 Backstrom L & Kantero R L Cross sectional studies of height and weight in Finnish children aged from birth to 20 years *Acta Paediatr Scand* Suppl 2?0 9 1971
3 Bayley N & Pinneau S R Tables for predicting adult height from skeletal age revised for use with the Greulich Pyle hand standards *J Pediatr 40* 423 195?
4 Greulich W W & Pyle I S *Radiographic Atlas of Skeletal Development of the Hand and Wrist* Stanford University Press Stanford 1959 2nd ed
5 Hallman N Backstrom Jarvinen L Kantero R L & Tiisala R Finnish centre for study in child growth and development *Ann Paediatr Fenn 12* 8 1966
6 Kantero R L Tiisala R & Backstrom L Predictability of individual height and weight growth from cross sectional distance curves from birth to 10 years *Acta Paediatr Scand* Suppl 2 0 33 1971
7 Perheentupa J Lenko H L Nevalainen I Nuutymaki M Sodeholm A & Taipale V Hormonal therapy in Turner s syndrome Growth and psychological aspects In *Proceedings of the XIV International Congress of Pediatrics 5* Buenos Aires 1971 p 121
8 Roche A F & Wettenhall H N B The prediction of adult stature in tall girls *Aust Paediatr J 5* 13 1969
9 Roche A F Wainer H & Thissen D The RWT method for the prediction of adult stature *Pediatrics 56* 10?6 1975
10 Roche A F Wainer H & Thissen D *Skeletal maturity The knee joint as a biological indicator* Plenum Medical Book Company New York 1976

11 Tanner J M *Growth at adolescence* Blackwell Scientific Publications Oxford 1962 2nd ed
12 Tanner J M Whitehouse R H Marshall W A & Carter B S Prediction of adult height from height bone age and occurrence of menarche at ages 4 to 16 with allowance for midparent height *Arch Dis Child 50* 14 1975
13 Tanner J M Whitehouse R H Marshall W A Healy M J R & Goldstein H *Assessment of skeletal maturity and prediction of adult height TW 2 Method* Academic Press New York 1975
14 Walker R N Standards for somatotyping children 1 The prediction of young adult height from children s growth data *Ann Hum Biol 1* 14 1974
15 Zachmann M Ferrandez A Murset G & Prader A Estrogen treatment of excessively tall girls *Helv Paediatr Acta 30* 11 1975
16 Zachmann M Ferrandez A Murset G Gnehm H E & Prader A Testosterone treatment of excessively tall boys *J Pediatr* 88 116 1976

Submitted April 20 1978
Accepted July 7 1978

(H L L) Children s Hospital
University of Helsinki
SF 00290 Helsinki 29
Finland

Acta Pædiatr Scand 68 93–96 1979

SERIAL DETERMINATIONS OF SERUM FERRITIN IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA

Evaluation of its Usefulness as a Prognostic Index

M E KOLLER[1] I ROMSLO P H FINNE and B HANEBERG

From the [1]Department of Clinical Biochemistry and the Department of Paediatrics University of Bergen Bergen Norway

ABSTRACT Koller M E Romslo I Finne P H and Haneberg B (Laboratory of Clinical Biochemistry Department of Paediatrics University of Bergen Bergen Norway) Serial determinations of serum ferritin in children with acute lymphoblastic leukemia Acta Paediatr Scand 68 93 1979 —Thirty children with acute lymphoblastic leukemia were monitored with serial serum ferritin determinations for up to 17 months In children with acute lymphoblastic leukemia before initiation of therapy or in relapse the mean serum ferritin concentration was 636 μg/l In children who went into primary remission the mean serum ferritin concentration fell from 265 μg/l prior to start of treatment to 161 μg/l after 3 months of treatment Five patients relapsed Their serum ferritin levels prior to the relapses ranged from 7 to 135 μg/l At the time of relapse a further increase in serum ferritin was found in only 2 of the children Thus whereas high serum ferritin levels may signal disease activity in acute lymphoblastic leukemia a normal serum ferritin level does not exclude disease activity or impending relapse

KEY WORDS Acute lymphoblastic leukemia serum ferritin

Determination of serum ferritin is a simple way of quantifying the body iron stores in normal subjects and in patients with iron deficiency or iron overload (1 5 7 10) However in a number of conditions a marked increase in serum ferritin occurs that is unrelated to the iron stores Thus high levels inappropriate for the amount of iron storage have been found in patients with liver diseases tumour bearing patients and in patients with leukemia (2 4 6–8) It has been suggested that the high concentrations of ferritin in these patients are related to an abnormal production and release of ferritin together with reduced plasma clearance (2)

This report deals with serial determinations of serum ferritin in infants and children with acute lymphoblastic leukemia during a 17 month period Data on serial determinations of ceruloplasmin orosomucoid and alanine aminotransferase activity are also included The aim of the present study was to evaluate the usefulness of the serum ferritin concentration as a prognostic index in childhood leukemia

MATERIALS AND METHODS

Sera were obtained at monthly intervals for up to 17 months from 30 infants and children with acute lymphoblastic leukemia (ALL) The following patients were studied

8 girls and 5 boys aged 5–15 years with ALL in hematological remission after cessation of therapy On entering the study they had been off therapy for from 4 months to 4 years

5 girls and 12 boys aged 8 months–13 years with ALL on therapy Twelve of these patients were studied from the time of diagnosis i e before treatment Four patients entered the study during treatment while in hematological remission and 1 entered the study while in relapse

Conventional chemotherapy was used Induction of remission in ALL was usually achieved by vincristine and prednisone or dexamethasone followed by asparaginase and lately by high doses of methotrexate intravenously Maintenance therapy with 6-mercaptopurine plus methotrexate and reinduction courses of vincristine and steroids every 2–3 months were continued for 3 years In cases of relapse cytosine arabinoside and cyclophosphamide were added All patients received methotrexate intrathecally

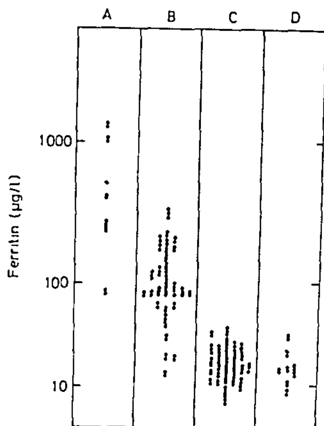


Fig 1 Serum ferritin concentrations in children with acute lymphoblastic leukemia (*A*) before treatment and on treatment while in incomplete remission or relapse (*B*) on therapy while in remission (*C*) after cessation of therapy The concentrations in healthy children (*D*) are also shown

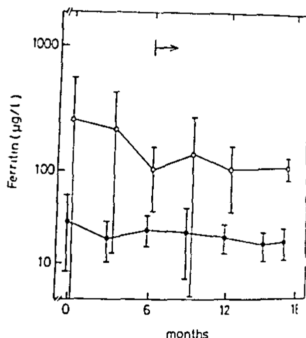


Fig 2 Serial determinations of serum ferritin in 13 long term survivors of childhood acute lymphoblastic leukemia off therapy (●) and in 10 children with acute lymphoblastic leukemia responding favourably to treatment at presentation and during treatment (O) The results are given mean±1 S D At the point indicated (*arrow*) all the patients on treatment had normal bone marrow smear

Radiation therapy was restricted to patients with leukemic involvement of the central nervous system or with testicular infiltrations

The serum concentrations of ferritin were measured by a solid phase radioimmunoassay kit (from Ramco Lab Inc Houston Texas USA) using antihuman spleen ferritin This kit has been shown to give values slightly lower than those obtained by other methods (M Worwood personal communication)

Ceruloplasmin and orosomucoid were determined by standard immunoplate techniques (Behringwerke AG Marburg Lahn W Germany)

Alanine aminotransferase (ALAT) was determined by the method recommended by The Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology (3) All determinations were run in duplicate

Our reference values (mean±2 S D) determined on 31 healthy children between 2–14 years of age were 3–31 µg/l 0 20–0 40 g/l 0 40–0 80 g/l and 20–40 U/l for ferritin ceruloplasmin orosomucoid and ALAT respectively The day to day coefficients of variation of the determinations were ferritin 6 9 ceruloplasmin 7 0 orosomucoid 7 7 and ALAT 6 0%

RESULTS

In patients with pathological marrow smears either on or off therapy the mean serum ferritin concentration was 636 µg/l (range 49–4945 µg/l from 38 observations in 16 patients) (Fig 1) On the other hand in patients in remission and with a normal bone marrow the mean serum ferritin concentration was 133 µg/l (range 7–610 µg/l from 97 observations in 13 patients) Children previously treated for ALL and still in remission had mean serum ferritin concentration 23 µg/l (range 4–100 µg/l from 96 observations in 13 patients)

Among children who responded favourably to therapy ($n=10$) the remission coincided with a fall in the serum ferritin concentration (Fig 2) The mean pretreatment value was 265 µg/l (range 49–1300) and after 3 months of successful therapy the mean value was 161 µg/l (range 35–364 µg/l) Corresponding

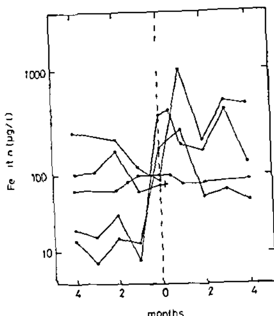


Fig 3 Serial determination of serum ferritin in children with acute lymphoblastic leukemia in remission and during relapse At the point indicated (stippled line) the patients were in relapse with pathological bone marrow

figures for ALAT were 33 and 66 U/l respectively

The serum ferritin concentrations in 5 patients who relapsed did not increase prior to the appearance of pathological bone marrow (Fig 3)

The serum ferritin concentration showed small fluctuations during infections and did not correlate with the concentrations of orosomucoid and ceruloplasmin Furthermore there was no correlation between serum ferritin and ALAT (data not shown)

DISCUSSION

The high serum ferritin concentrations found in children with ALL before treatment or during relapse are in agreement with previous reports (4 6 8) The values were 10–30 times higher than those found in healthy children (7) On the other hand our results in children in remission on therapy differ from those reported by Parry et al (6) who found a marked increase in serum ferritin concentrations during induction of primary remission Our findings suggest that following chemotherapy an uneventful remission coincides with a fall in serum ferritin concentration This is also supported by the findings of Siimes et al (8) who found that the serum ferritin values in children with ALL during remission were significantly lower than at initial diagnosis

It should be noted that increasing serum ferritin levels did not precede relapse of ALL In fact increasing serum ferritin levels were not found until after the relapse had been diagnosed by conventional marrow smears

Such hyperferritinemia might be ascribed to an increased ferritin synthesis by the leukemic cells (11) together with an increased release from damaged leukemic cells (6) This assumption has been challenged by Siimes et al (8) who found no increase in the serum ferritin on rapid lysis of leukemic cells following chemotherapy Our results favour those reported by Siimes et al (8)

In liver diseases hyperferritinemia has been ascribed to decreased removal of ferritin from the circulation by damaged hepatic parenchymal cells (2 8) As shown by Parry et al (6) and confirmed in the present study in leukemia there was no correlation between serum ferritin concentration and transaminase activity i e the high ferritin levels did not result from liver damage

Serum ferritin is frequently elevated in infections and inflammations irrespective of the iron status of the patient (5 7) This may suggest that the hyperferritinemia in ALL could be an acute phase reaction (8 9) However the fact that our results fail to show any correlation between serum ferritin concentration and ceruloplasmin or orosomucoid in children with ALL would suggest that the high ferritin levels are not the results of an acute phase reaction

Our findings indicate that in patients with ALL determination of serum ferritin may be useful in assessing the state of remission but

it is of dubious value in the early detection of relapses

ACKNOWLEDGEMENTS

The technical assistance of Mrs Nina Gade Christensen is gratefully acknowledged The study was supported in part by the Norwegian Research Council for Science and the Humanities

REFERENCES

1 Jacobs A Miller F Worwood M Beamish M R & Wardrop C A Ferritin in the serum of normal subjects and patients with iron deficiency and iron overload *Br Med J IV* 206 1972
2 Jacobs A Serum ferritin and iron stores *Fed Proc 36* 2024 1977
3 Keiding R Horder M Gerhardt W Pitkanen E Tenhunen R Stromme J H Theodorsen L Waldenstrom J Tryding N & Westlund L Recommended methods for the determination of four enzymes in blood *Scand J Clin Lab Invest 33* 291 1974
4 Jones P A E Miller F M Worwood M & Jacobs A Ferritinaemia in leukemia and Hodgkin s disease *Br J Cancer 27* 212 1973
5 Lipschitz D A Cook J D & Finch C A A clinical evaluation of serum ferritin as an index of iron stores *N Engl J Med 290* 1213 1974
6 Parry D H Worwood M & Jacobs A Serum ferritin in acute leukemia at presentation and during remission *Br Med J I* 245 1974
7 Siimes M A Addiego J E & Dallman P R Ferritin in serum Diagnosis of iron deficiency and iron overload in infants and children *Blood 43* 581 1974
8 Siimes M A Wang W C & Dallman P R Elevated serum ferritin in children with malignancies *Scand J Haematol 19* 153 1977
9 Thomas W J Koenig H M Lightsey jr A L & Green R Free erythrocyte porphyrin hemoglobin ratios serum ferritin and transferrin saturation levels during treatment of infants with iron-deficiency anemia *Blood 49* 455 1977
10 Walters G O Miller F M & Worwood M Serum ferritin concentration and iron stores in normal subjects *J Clin Path 26* 770 1973
11 Worwood M Summers M Miller F Jacobs A & Whittaker J A Ferritin in blood cells from normal subjects and patients with leukemia *Br J Haematol 28* 27 1974

Submitted April 11 1978
Accepted June 12 1978

(M E K) Laboratory of Clinical Biochemistry
University of Bergen
Bergen
Norway

Acta Pædiatr Scand 68 97–101 1979

INFLUENCE OF GENERAL ANAESTHESIA ON ANO RECTAL MANOMETRY IN HEALTHY CHILDREN

B FRENCKNER and M L MOLANDER

From the Department of Paediatric Surgery St Goran s Hospital Stockholm Sweden

ABSTRACT Frenckner B & Molander M.-L. (Th Department of Paediatric Surgery St Goran s Hospital Stockholm Sweden) Influence of general anaesthesia on ano-rectal manometry in healthy children Acta Paediatr Scand 68 97 1979.—According to several investigations ano-rectal manometry is a valuable diagnostic test of Hirschsprung s disease In order to yield accurate results it requires a quiet calm child who cooperates In the few instances when this is not possible general anaesthesia may be desirable Manometric recordings of the internal anal sphincter activity were therefore performed in 15 healthy children when awake and during general anaesthesia The tonic activity at rest was significantly redu ed during anaesthesia Relaxations of the internal sphincter in response to rectal distension were recorded in all children both when awake and during anaesthesia They were however significantly less pronounced during anaesthesia These findings strongly suggest that ano-rectal manometry in the diagnosis of Hirschsprung s disease may be performed with advantage during general anaesthesia if the child does not cooperate when awake

KEY WORDS Ano-rectal manometry Hirschsprung s disease general anaesthesia

Like other smooth muscle the internal anal sphincter is in a state of continuous tonic activity and normally keeps the anal canal closed When the rectum is distended however there is a relaxation of this sphincter (2 9 13) which results in a fall in anal pressure This rectosphincteric reflex is mediated via local nerve pathways in the wall of the gut and is independent of spinal cord connections (2) When the ganglion cells of the rectum are absent however as in Hirschsprung s disease the internal sphincter fails to relax upon rectal distension (11 12) Several authors have found this to be a valuable diagnostic test of Hirschsprung s disease (1 6 10 14 15 16) To obtain conclusive recordings it is essential that the patient is quiet and calm which is mostly the case If the child does not cooperate however general anaesthesia may be desirable but it is not yet known how this can influence the normal rectosphincteric reflexes

The aim of the present investigation was to study the influence of general anaesthesia on anal pressure and rectosphincteric reflexes in healthy children

MATERIAL

The study was undertaken on 15 healthy children admitted to the hospital for a minor operation such as circumcision or herniectomy None of them had any history of intestinal or ano-rectal disorders The children were divided into three groups according to age Group one 0–1 years of age (mean 4 months range 2–10) *group two* 5–7 years (mean 6) and group three 10–12 years (mean 11) Each group consisted of 5 children

METHODS

Equipment

Anal pressure was recorded at the level of the internal sphincter using a cuff of a Portex *endotracheal tube* no 5 0 The tube was given a total length of 13 14 *cm by* cutting off the outer end For children below one year of age the cuff was tied off with a silk ligature to *reduce its length* to 1–1½ cm The cuff was *then filled with* water and connected via a thin polyethylene tube to the recording equipment The amount of water *in the cuff* was *adjusted* so that the cuff was expanded but the pressure inside *it did not* exceed zero

Rectal distension was achieved with a latex balloon When empty this *measured* 1 5–? 0 cm It was connected to a polyethylene tube about 50 cm long Its internal *diameter was* 0 mm and its external 2 4 mm This tube passed through *the endotracheal* tube and led to the recording equipment via a *three way* stopcock through which air could be inflated into the balloon (Fig 1)

The recording equipment comprised a pressure trans

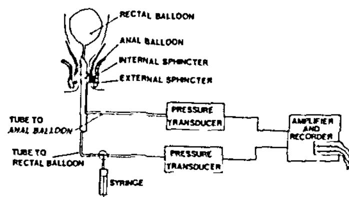


Fig 1 Diagrammatic representation of the method with an anteroposterior view of the rectal and anal balloons in situ

ducer (Statham P23) amplifier (Grass 7 P1) and recorder (Grass 7) connected separately to each line from the rectal balloon and the anal cuff The same technique has been used earlier (6)

Procedure

The children below one year of age were lying on their backs with hips and knees in flexion the older ones on their left side also with a flexion in hips and knees The rectal balloon was placed in position with the aid of some exploration cream and a swab To ensure that the balloon lay unfolded it was inflated with 5–10 ml of air which was immediately evacuated The anal balloon (i e the cuff of the endotracheal tube) was then placed just inside the anal verge with the tube to the rectal balloon running through it (Fig 1) An assistant held this balloon in position during the examination

Anal pressure was then recorded continuously When the child was calm and the pressure stabilized the rectal balloon was inflated with air 5 and 10 ml in group one 10 and 20 ml in group two and 20 and 50 ml for the children in group three Each inflation took less than 0 5 secs and the rectal balloon was kept filled for about 10–20 sec before it was evacuated This was repeated several times

Each child was examined twice the first time awake and the second time during general anaesthesia When examined awake the infants (i e group one) were premedicated with diazepam (Valium Roche) 0 3 mg/kg body weight given orally one hour before the examination The older children were not premedicated The children were kept calm by the mother and in the case of the infants also with the aid of a feeding bottle or a dummy When examined during anaesthesia the children were premedicated with morphine and atropine (Table 1) one hour before the examination General anaesthesia was achieved with thiomebumal sodium (Pentothal Abbott) about 5 mg/kg body weight given intravenously and with inhalation of 80% nitrous oxide in oxygen

Statistics

Standard statistical procedures were used Means of two groups were compared with Student s paired *t* test (3) When comparing means of three groups one way analysis of variance was used (3) Data in the text and table are given as mean±S E (standard error of the mean)

RESULTS

All 15 children examined in this investigation were quiet calm and cooporated well when awake Conclusive recordings were thus obtained from all of them

Anal pressure

Anal pressure at rest (Table 2) when the children were awake was significantly ($p<0\ 05$) lower in group one (5 7±0 75 kPa 43±5 6 mmHg) than in group two (9 5±0 29 kPa 71±2 2 mmHg) and in group three (10 1±0 68 kPa 76±5 1 mmHg) During general anaesthesia it decreased in all groups among infants i e group one from 5 7±0 75 kPa (43±5 6 mmHg) to 4 7±0 63 kPa (35±4 7 mmHg) which is not fully significant In group two it decreased from 9 5±0 29 kPa (71±2 2 mmHg) to 6 0±0 44 kPa (45±3 3 mmHg) and in group three from 10 1±0 68 kPa (76±5 1 mmHg) to 6 5±0 48 kPa (49±3 6 mmHg) which are both significant ($p<0\ 05$) differences

Table 1 *Dose of morphine and atropine given as premedication before general anaesthesia*

Body weight (kg)	Morphine (mg)	Atropine (mg)
3	0 1	0 05
4	0 1	0 05
5	0 15	0 1
6	0 2	0 1
7	0 3	0 2
8	0 4	0 2
9	0 5	0 2
10	0 6	0 2
12	1 0	0 2
14	1 5	0 3
16	2 2	0 3
18	2 6	0 3
20	3 8	0 3
22	4 5	0 5
24	5 3	0 5
26	6 0	0 5
28	6 8	0 5
30	7 5	0 5

Table 2 Anal pressure at rest (kPa) and relaxations (kPa) of the internal anal sphincter at different rectal volumes in the three groups of children when awake and under general anaesthesia (GA) respectively

	Anal pressure	Relaxations 10 ml	20 ml	50 ml
Group 1				
Awake	5 7±0 75	3 5±0 40	–	–
GA	4 7±0 63	2 7±0 45		
Group 2				
Awake	9 5±0 79	3 1±0 40	3 5±0 79	–
GA	6 0±0 44	1 7±0 48	1 7±0 36	–
Group 3				
Awake	10 1±0 68	–	4 3±1 04	5 6±0 69
GA	6 5±0 48	–	2 9±0 73	3 5±0 56

denotes a significant difference (p<0 05) between the two conditions

Relaxation

Relaxations of the internal sphincter were recorded in all children both when awake and during general anaesthesia (Fig 2) The exact manner in which they were measured is indicated in Fig 2 i e the least anal pressure recorded during a relaxation was subtracted from the resting anal pressure the difference being regarded as the relaxation

In general the relaxations were less pronounced during anaesthesia (Table 2) In group one when the rectal balloon was inflated with 10 ml they averaged 3 5±0 40 kPa (26±3 0 mmHg) when awake and 2 7±0 45

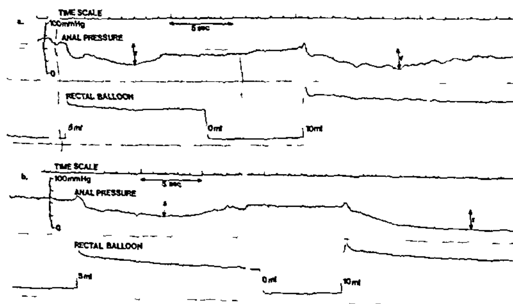


Fig 2a Recording of anal pressure during rectal distension in a 4 month-old boy when awake In response to each rectal inflation there is a relaxation of the internal sphincter indicated with "r"

Fig 2b Recording of anal pressure during rectal distension in the same child but during general anaesthesia Anal pressure at rest is slightly lower and the relaxations are slightly smaller

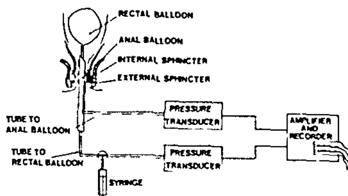


Fig 1 Diagrammatic representation of the method with an anteroposterior view of the rectal and anal balloons in situ

ducer (Statham P23) amplifier (Grass 7 P1) and recorder (Grass 7) connected separately to each line from the rectal balloon and the anal cuff The same technique has been used earlier (6)

Procedure

The children below one year of age were lying on their backs with hips and knees in flexion the older ones on their left side also with a flexion in hips and knees The rectal balloon was placed in position with the aid of some exploration cream and a swab To ensure that the balloon lay unfolded it was inflated with 5–10 ml of air which was immediately evacuated The anal balloon (i e the cuff of the endotracheal tube) was then placed just inside the anal verge with the tube to the rectal balloon running through it (Fig 1) An assistant held this balloon in position during the examination

Anal pressure was then recorded continuously When the child was calm and the pressure stabilized the rectal balloon was inflated with air 5 and 10 ml in group one 10 and 20 ml in group two and 20 and 50 ml for the children in group three Each inflation took less than 0 5 secs and the rectal balloon was kept filled for about 10–20 sec before it was evacuated This was repeated several times

Each child was examined twice the first time awake and the second time during general anaesthesia When examined awake the infants (i e group one) were premedicated with diazepam (Valium Roche) 0 3 mg/kg body weight given orally one hour before the examination The older children were not premedicated The children were kept calm by the mother and in the case of the infants also with the aid of a feeding bottle or a dummy When examined during anaesthesia the children were premedicated with morphine and atropine (Table 1) one hour before the examination General anaesthesia was achieved with thiomebumal sodium (Pentothal Abbott) about 5 mg/kg body weight given intravenously and with inhalation of 80% nitrous oxide in oxygen

Statistics

Standard statistical procedures were used Means of two groups were compared with Student s paired *t* test (3) When comparing means of three groups one way analysis of variance was used (3) Data in the text and table are given as mean±S E (standard error of the mean)

RESULTS

All 15 children examined in this investigation were quiet calm and cooporated well when awake Conclusive recordings were thus obtained from all of them

Anal pressure

Anal pressure at rest (Table 2) when the children were awake was significantly ($p<0\ 05$) lower in group one (5 7±0 75 kPa 43±5 6 mmHg) than in group two (9 5±0 29 kPa 71±2 2 mmHg) and in group three (10 1±0 68 kPa 76±5 1 mmHg) During general anaesthesia it decreased in all groups among infants i e group one from 5 7±0 75 kPa (43±5 6 mmHg) to 4 7±0 63 kPa (35±4 7 mmHg) which is not fully significant In group two it decreased from 9 5±0 29 kPa (71±2 2 mmHg) to 6 0±0 44 kPa (45±3 3 mmHg) and in group three from 10 1±0 68 kPa (76±5 1 mmHg) to 6 5±0 48 kPa (49±3 6 mmHg) which are both significant ($p<0\ 05$) differences

Table 1 *Dose of morphine and atropine given as premedication before general anaesthesia*

Body weight (kg)	Morphine (mg)	Atropine (mg)
3	0 1	0 05
4	0 1	0 05
5	0 15	0 1
6	0 2	0 1
7	0 3	0 2
8	0 4	0 2
9	0 5	0 2
10	0 6	0 2
12	1 0	0 2
14	1 5	0 3
16	2 2	0 3
18	2 6	0 3
20	3 8	0 3
22	4 5	0 5
24	5 3	0 5
26	6 0	0 5
28	6 8	0 5
30	7 5	0 5

Table 2 *Anal pressure at rest (kPa) and relaxations (kPa) of the internal anal sphincter at different rectal volumes in the three groups of children when awake and under general anaesthesia (GA) respectively*

	Anal pressure	Relaxations 10 ml	20 ml	50 ml
Group 1				
Awake	5 7±0 75	3 5±0 40	–	–
GA	4 7±0 63	2 7±0 45		
Group 2				
Awake	9 5±0 79	3 1±0 40	3 5±0 79	–
GA	*6 0±0 44*	*1 7±0 48*	1 7±0 36	–
Group 3				
Awake	10 1±0 68	–	4 3±1 04	5 6±0 69
GA	6 5±0 48	–	2 9±0 73	3 5±0 56

denotes a significant difference ($p < 0.05$) between the two conditions

Relaxation

Relaxations of the internal sphincter were recorded in all children both when awake and during general anaesthesia (Fig 2) The exact manner in which they were measured is indicated in Fig 2 i e the least anal pressure recorded during a relaxation was subtracted from the resting anal pressure the difference being regarded as the relaxation

In general the relaxations were less pronounced during anaesthesia (Table 2) In group one when the rectal balloon was inflated with 10 ml they averaged 3 5±0 40 kPa (26±3 0 mmHg) when awake and 2 7±0 45

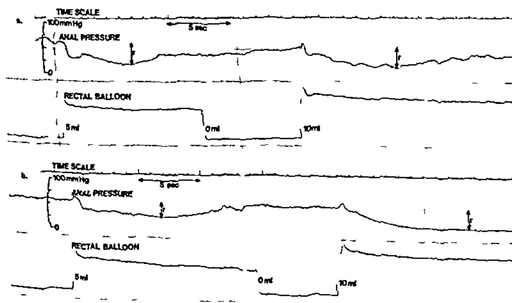


Fig 2a Recording of anal pressure during rectal distension in a 4 month-old boy when awake In response to each rectal inflation there is a *relaxation* of the internal sphincter indicated with *r*

Fig 2b Recording of anal pressure during rectal distension in the same child but during general anaesthesia Anal pressure at rest is slightly lower and the relaxations are slightly smaller

kPa (20±3 4 mmHg) during anaesthesia the difference being significant ($p<0$ 05) In group two the relaxations decreased from 3 1±0 40 kPa (23±3 0 mmHg) to 1 7±0 48 kPa (13±3 6 mmHg) with 10 ml in the rectal balloon and from 3 5±0 79 kPa (26±5 9 mmHg) to 1 7±0 36 kPa (13±2 7 mmHg) with 20 ml in the rectal balloon the first difference being *significant ($p<0$ 05)* Among the oldest children i e group three the relaxations decreased from 4 3±1 04 kPa (32±7 8 mmHg) to 2 9±0 73 kPa (22±5 5 mmHg) with 20 ml in the rectal balloon and from 5 6±0 69 kPa (42±5 2 mmHg) to 3 5±0 56 kPa (26±4 2 mmHg) ($p<0$ 05) with 50 ml in the rectal balloon

Defecation

Four of the 5 infants in group one defecated during the examination when awake This was preceded by a short period of very low anal pressure Reflex defecation during the manometry did not occur in any of the older children nor did it occur in any child during general anaesthesia

DISCUSSION

The anal canal is surrounded by the internal and external anal sphincters which both exhibit continuous tonic activity at rest The pressure which can be recorded in the anal canal at rest is generated predominantly by the internal sphincter but to some extent by the external sphincter (4 7) Consequently the decreased anal pressure observed in this investigation during general anaesthesia may theoretically be caused by a decreased activity in either sphincter If it were due only to a lowered external sphincter activity however the recorded relaxation of the internal sphincter should not be reduced, as it was in this study In fact a slight increase would be expected as the inflation reflex of the external sphincter i e reflex contraction in response to rectal distension (8) normally counteracts the internal sphincter relaxation (7) It is therefore concluded that general anaesthesia with thiomebumal to some extent inhibits the tonic activity of the internal anal sphincter

In response to rectal distension relaxations of the internal sphincter were recorded in all children both when awake and during general anaesthesia though the relaxations were significantly ($p<0$ 05) less pronounced during anaesthesia This reduction is explained by the inhibited tonic activity of the internal sphincter at rest induced by thiomebumal as discussed above

In other words the general anaesthesia inhibits the tonic activity of the internal anal sphincter and reduces the relaxation in response to rectal distension in healthy children This rectosphincteric reflex is however still present and is not qualitatively affected by the general anaesthesia The lack of internal sphincter relaxation in patients with Hirschsprung s disease is caused by the absence of ganglion cells and may therefore not be affected by general anaesthesia Consequently anorectal manometry in the diagnosis of Hirschsprung s disease may be performed during general anaesthesia with thiomebumal if the child does not cooperate when awake

Anal pressure at rest with the child awake was significantly ($p<0$ 05) lower in group one (5 7±0 75 kPa 43±5 6 mmHg) than in groups two (9 5±0 29 kPa 71±2 2 mmHg) and three (10 1±0 68 kPa 76±5 1 mmHg) The latter two groups did not differ essentially from adults in this respect (8 5±0 47 kPa 64±3 5 mmHg $n=10$) (7) This may indicate that anal sphincter activity is lower in infants than in older children and that it seems to increase and reach adult values after a few years of life i e when anal continence normally develops However the absolute values may to some extent also have been influenced by the smaller size of the anal canal in infants but this possible source of error was partly compensated for by the shorter anal cuff that was used in this group

The diagnosis of Hirschsprung s disease can

usually be established with the aid of barium enema examination and conventional rectal biopsy The interpretation of X rays findings may however be difficult in some patients and rectal biopsy is not without complications and mortality (5) Consequently ano rectal manometry is a valuable complement in the diagnosis as several studies show that its reliability in this context is high (1 6 10 14 15 16) Conclusive recordings require a quiet calm child which is mostly the case In the few instances however when the patient does not cooperate it is suggested on the basis of the present investigation that ano-rectal manometry may be performed with advantage during general anaesthesia

REFERENCES

1 Aaronson I & Nixon H H A clinical evaluation of ano-rectal pressure studies in the diagnosis of Hirschsprung s disease *Gut 13* 138 1972

2 Denny Brown D & Robertson E G An investigation of the nervous control of defecation *Brain 58* 256 1935

3 Dixon W D & Massey F J *Introduction to Statistical Analysis* McGraw Hill New York 1957

4 Duthie H L & Watts J M Contribution of the external anal sphincter to the pressure zone in the anal canal *Gut 6* 64 1965

5 Fraser G C & Wilkinson A W Neonatal Hirschsprung s disease *Br Med J iii* 7 1967

6 Frenckner B Ano-rectal manometry in the diagnosis of Hirschsprung s disease in infants *Acta Paediatr Scand* 67 187 1978

7 Frenckner B & von Euler Chr Influence of pudendal block on the function of the anal sphincters *Gut 16* 482 1975

8 Gaston E A The physiology of fecal continence *Surg Gynecol Obstet 87* 280 1948

9 Gowers W R The autonomic action of the sphincter ani *Proc R Soc Lond 26* 77 1877

10 Holschneider A M Moderne Funktionsdiagnostik anorectaler Erkrankungen im Kindesalter *Munch Med Wochenschr 116* 1179 1974

11 Lawson J O N & Nixon H H Anal canal pressure in the diagnosis of Hirschsprung s disease *J Pediatr Surg 2* 544 1967

12 Schnaufer L Talbert J L Haller J A Reid N C R W Tobon F & Schuster M M Differential sphincteric studies in the diagnosis of ano-rectal disorders of childhood *J Pediatr Surg 2* 538 1967

13 Schuster M M Hendrix T R & Mendeloff A I The internal anal sphincter response Manometric studies on its normal physiology neural pathways and alteration in bowel disorders *J Clin Invest 42* 196 1963

14 Tobon F Reid N C R W Talbert J L & Schuster M M Nonsurgical test for the diagnosis of Hirschsprung s disease *N Engl J Med 278* 188 1968

15 Varma K K & Stephens D Neuromuscular reflexes of rectal continence *Aust NZ J Surg 41* 263 1972

16 Verder H Johansen K S & Engbaek K Anal tonometry A diagnostic help in Hirschsprung s disease *Acta Paediatr Scand 62* 59 1973

Submitted April 18 1978
Accepted June 15 1978

(B F) Department of Paediatric Surgery
St Gorans sjukhus
Box 12500
S 11281 Stockholm
Sweden

Acta Pædiatr Scand 68 103–108 1979

ASSESSMENT OF LUNG FUNCTION ON HEALTHY CHILDREN USING AN ELECTRONIC SPIROMETER AND AN AIR FLOWMETER BEFORE AND AFTER INHALATION OF AN ADRENERGIC RECEPTOR STIMULANT

GUNNAR DALÉN and BENGT KJELLMAN

From the Department of Paediatrics Karnsjukhuset Skovde Sweden

ABSTRACT Dalen G and Kjellman B (Department of Paediatrics Karnsjukhuset Skovde Sweden) Assessment of lung function on healthy children using an electronic spirometer and an Air flowmeter before and after inhalation of an adrenergic receptor stimulant Acta Paediatr Scand 68 103 1979.—VC measured with a Monaghan electronic spirometer equipped with a backflow valve is significantly lower (about 4%) than when measured with the same spirometer without such a valve The measurements of $FEV_{1.0}$ were not influenced by the valve 73 healthy children were investigated with the Monaghan spirometer equipped with the backflow valve and normal reference data were established The results were very similar to those obtained in an investigation of healthy children with the same spirometer about one year earlier Reference data on children for a simple flow meter Airflometer (Glaxo Ltd) are given The data correlated very highly to the $FEV_{1.0}$ values obtained by the Monaghan spirometer After inhalation of salbutamol healthy children had a small and significant increase of FEV_1 and of the Airflometer value but not of VC The deviations of the differences were small A 6% increase of VC and 10% increase of $FEV_{1.0}$ were taken as normal upper limits after inhalation of salbutamol Corresponding increase of the Airflometer values was 15 arbitrary units for children with body heights 116–145 cm and 21 units for children with body heights 146–175 cm

KEY WORDS Children spirometry electronic spirometer Airflometer salbutamol inhalation

A desirable increase of pulmonary function tests on children with bronchial or lung diseases requires equipment with such characteristics as simple operation and fast delivery of data The equipment must also be reliable and it is important that evaluation of it is done with regard to precision and accuracy and it is also important to establish reference data on healthy subjects

In a previous study (3) on healthy children we compared two electronic spirometers (Spirotron Drager and Monaghan M 403) and one wedge bellows spirometer (Vitalograph) with a water spirometer The Monaghan spirometer was used without the backflow valve which may have presented the risk of getting erroneously high values of vital capacity (VC) The comparison showed encouraging results but further evaluation of the spirometers was suggested

A new simple flow meter Airflometer (AFM Glaxo Ltd) has shown good performance in studies on adults and in phantom investigations (1 6 8) A high correlation of the AFM values was found expressed in arbitrary units (AFM units) to the forced expiratory volume in one second ($FEV_{1.0}$) obtained in spirometry These results have encouraged us to the present evaluation of the AFM on children

The aims of the present study are

1 to compare the values given by a Monaghan spirometer with its backflow valve and without it

Table 2 *Vital capacity (VC) forced expiratory volume in one second ($FEV_{1\,0}$) in relation to body height in metres (H)*

There are given data from Monaghan spirometry with a backflow valve (M 1) i e the present investigation data of Monaghan spirometry without a valve (M 2) and data of Bernstein spirometry (B) The data of M 2 and B were obtained in a previous investigation (3) Standard deviations (SD) are expressed in litres and in percentages of the mean VC or mean FEV

Lung function	Spiro-meter	*n*	Equation of the regression lines	S D l	S D %	Coefficient of correlation
VC boys	M 1	34	$0\ 78? \times H^3 + 0\ 077$	0 33	12	0 98
	M ?	31	$0\ 833 \times H\ - 0\ 001$	0 ?8	11	0 98
	B	31	$0\ 781 \times H^3 + 0\ 13?$	0 27	11	0 98
VC girls	M 1	39	$0\ 759 \times H^3 + 0\ 000$	0 ?4	10	0 98
	M ?	30	$0\ 9?6 \times H^3 - 0\ 464$	0 27	11	0 97
	B	30	$0\ 768 \times H\ - 0\ 039$	0 ?4	10	0 96
FEV boys	M 1	34	$0\ 6?5 \times H^3 + 0\ 244$	0 26	11	0 98
	M ?	31	$0\ 65? \times H^3 + 0\ 159$	0 ?1	10	0 98
	B	31	$0\ 671 \times H^3 + 0\ 100$	0 21	10	0 98
FEV girls	M 1	39	$0\ 700 \times H\ - 0\ 058$	0 ?2	10	0 98
	M ?	30	$0\ 710 \times H^3 - 0\ 108$	0 18	9	0 97
	B	30	$0\ 690 \times H^3 - 0\ 059$	0 19	9	0 97

without a backflow valve showed significantly higher mean VC in spirometry without a valve (Table 1) No systematic difference was found for $FEV_{1\,0}$ Table 2 shows the equations of the regression lines of VC and $FEV_{1\,0}$ with respect to body height (raised to the third power) for the 73 healthy children The S D around the lines and the correlation coefficients are also given The same data of our previous study about one year ago are presented for comparison

To illustrate the similarity of the reference data obtained with the Monaghan spirometer in the two studies the values are given for three body heights in Table 3 For comparison the reference data of four Swedish studies using water spirometers are also shown The Table shows that the differences between the water spirometers are larger than the differences between the two studies of the Monaghan spirometer

Table 4 shows the equations of the regres

Table 3 *Vital capacity (VC) and forced expiratory volume in one second ($FEV_{1\,0}$) in healthy children*

The results of Monaghan spirometry with a backflow valve and without such a valve are given for the body heights 130 cm 145 cm and 160 cm For comparison corresponding values of four Swedish studies on healthy children with water spirometers are also given

	VC (l)						FEV_1 (l)					
	130 cm		145 cm		160 cm		130 cm		145 cm		160 cm	
Spirometer	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Monaghan valve	1 80	1 67	? 46	? 31	3 ?8	3 10	1 6?	1 48	2 15	? 08	2 80	2 81
Monaghan no valve (3)	1 83	1 57	2 54	2 36	3 41	3 33	1 60	1 45	2 15	2 06	2 83	2 80
Bernstein (3)	1 85	1 65	2 51	? 30	3 33	3 11	1 57	1 46	2 14	2 05	2 85	2 77
Bernstein ( )	1 96	00	? 78	2 63	3 77	3 40	1 71	1 72	? 37	2 30	3 19	3 0?
Bernstein (5)*	1 65		2 ?3		? 93		1 4?		1 89		2 44	
Bernstein (7)*	1 65		?6		3 00		1 46		1 97		2 63	

This study gives the results at BTPS for boys and girls why the values given in this table are recalculated to ATPS

Table 1 *Differences of vital capacity (VC) and forced expiratory volume in one second ($FEV_{1.0}$) from Monaghan spirometry with and without a backflow valve in 12 subjects*

The differences are value from spirometry without a backflow valve minus the value from spirometry with a backflow valve The differences are expressed in litres and also as a precentage of mean VC (3 66 l) and mean $FEV_{1.0}$ (3 05 l)

Lung function	M	S D
VC	+0 14 (3 8%)*	0 12 (3 3%)
$FEV_{1.0}$	+0 02 (0 7%)	0 09 (3 1%)

=not significant $=p<0\ 01$

2 to assess on healthy children normal reference data of the Monaghan spirometer equipped with a backflow valve

3 to compare the data of Monaghan spirometry of the present investigation to those obtained in a study about one year ago (3)

4 to assess on healthy children normal reference data of the AFM and compare the data with $FEV_{1.0}$ obtained by the Monaghan spirometer

5 and to assess on healthy children the change of spirometric values and AFM units after inhalation of an adrenergic receptor stimulant

MATERIAL

Twelve young people (eight females and four males) free from cardiopulmonary disease Their range of ages was 10–25 years (median 15) and their range of body height was 130–181 cm (median 166) Healthy children (34 boys and 39 girls) with an age range of 7–16 years The range of body heights was for the boys 120–187 cm (median 145) and for the girls 119–176 cm (median 149)

The children were chosen from an ordinary school according to the same criteria described previously (3)

METHODS

Equipment The Monaghan M 403 is an electronic spirometer in which a heated thermistor is cooled by the exhaled air The spirometer was calibrated against the ATPS values of a Bernstein spirometer

The Airflometer (AFM) is a simple flowmeter in which the forced expirati of the subject drives a small plastic turbine which connected to a reduction gear and mechanical measuring device The result is given in arbitrary units (AFM units) and is affected by both ra and time of flow The same AFM was used during th whole study Each complete revolution of the indicato is equal to 100 AFM units

The Pari Inhalier Boy is a jet nebulizer of compresso type which was used for inhalations of salbutamol solution (5 mg per ml) The dosage used was 0 15 mg pe kg body weight

Procedure Monaghan spirometry was performed a described previously (3) AFM investigation was done with the subject in a sitting position The highest value of three determinations was chosen

Monaghan spirometry was performed on the 12 young people free from cardiopulmonary disease in duplicate investigations in one of which the spirometer was equipped with a backflow valve and the other without Every other child started with and every other without a valve

Monaghan spirometry and AFM investigation were done on the 73 children The spirometer was equipped with the backflow valve On 62 of these 73 children spirometry and AFM investigation were performed before and 20 min after inhalation of salbutamol solution Permission to give the inhalation was not granted for the remaining 11 children

RESULTS

An analysis of the paired differences ($n=12$) obtained in Monaghan spirometry with and

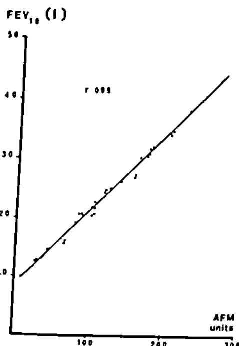


Fig 1 Forced expiratory volume in one second ($FEV_{1.0}$) expressed in l in relation to the results of Airflometer expressed in arbitrary units (AFM units) Number of children 73 The equation of the regression line is $FEV_{1.0}=AFM\times0\ 0129+0\ 857$ S D around the line is 0 14 l The coefficient of correlation (r) is 0 99

of VC probably depend upon the backflow valve in the way discussed above Other investigations of healthy Swedish children with water spirometers have however shown larger differences in results with one and the same type of spirometer i e water spirometers than those obtained by us on two different occasions with the Monaghan spirometer (2 3 5 7)

In our study a very high correlation (r= 0 99) was found between $FEV_{1.0}$ of the Monaghan spirometer and the AFM units which agrees with findings on children and adults (1 6 11) Previous phantom studies have also disclosed good precision in this simple instrument (1 8) The correlation to $FEV_{1.0}$ for the AFM should be better than for the Wright peak flow meter because not only the flow rate but also the time of flow influences the result To get measurable values of the AFM the subject must be capable of an exhalation corresponding to a $FEV_{1.0}$ of about one litre which is a drawback when investigating small children All of our children did however cooperate very satisfactory in the AFM maneuver and all of them gave measurable results The correlation of AFM to body height is as high as that of $FEV_{1.0}$ whereas the S D values around the lines are larger than those of $FEV_{1.0}$ which agrees with previous findings on adults (1) Previous studies have shown a high reproducibility for one and the same AFM whereas the variations sometimes are considerable among different apparatuses (1 8 11)

In proper spirometric investigations of subjects with bronchial diseases the tests should also be done after administration of a bronchodilating drug In Sweden inhalations of salbutamol solution with compressor nebulizers are often used as the first treatment of acute asthma and also in testing the bronchial reaction for diagnostic purposes The bronchial response of healthy children was not previously evaluated but it has often been said that an increase of $FEV_{1.0}$ of about 10% can be considered normal Souhrada & Buckley (9) state that significance is established only when the change in the spirometer values is 20% or greater In the study by Svenonius et al (10) 10 healthy children were investigated after exercise and inhalations of salbutamol There were no changes of mean VC or mean $FEV_{1.0}$ In our study of 62 healthy children there was no systematic change in mean VC after inhalation of salbutamol (Table 5) Furthermore the deviations of the VC differences are small and not greater than those found in an ordinary duplicate investigation with a water spirometer (4 5) There was however a systematic change of $FEV_{1.0}$ Despite the disturbing intermediate moment of inhalation the deviations of the $FEV_{1.0}$ differences were not greater than the deviations found in an ordinary duplicate investigation with a water spirometer (5)

We have chosen a 6% increase of VC and 10% increase of $FEV_{1.0}$ after administrations of salbutamol as normal upper limits which correspond to a value just above $M+2$ S D (Table 5)

Calculation of the change after inhalation of salbutamol in per cent is not possible for the AFM and the upper normal limits of change must be given in absolute AFM units An analysis of the change in relation to body height and original AFM values (Table 5) resulted in two limits an increase of 15 AFM units for the heights 116–145 cm and 21 AFM units for the heights 146–175 cm

ACKNOWLEDGEMENT

This investigation was supported by grants from Forenade Liv Mutual Life Insurance Company Stockholm and from Glaxo Lakemedel AB Goteborg Sweden

REFERENCES

1 Arborelius M & Christensson P Airflometer—en enkel apparat för lungfunktionstest *Lakartidn 75* 38 1978
2 Bjure J Spirometric studies in normal subjects IV Ventilatory capacities in healthy children 7–17 years of age *Acta Paediatr Scand 52* 232 1963
3 Dalen G & Kjellman B Evaluation of three spirometers on healthy children *Acta Paediatr Scand* in press

Table 4 *The relations of the Airflometer values (AFM) to body height (H) and forced expirator volume in one second ($FEV_{1.0}$) to AFM in healthy children*

The equations of linear regression the standard deviations (S D) and the coefficients of correlation (r) are giver AFM is expressed in arbitrary units $FEV_{1.0}$ in litres and H in metres

Regression	Equation	S D	r
AFM to H			
Boys	$AFM=45\ 78\times H^3-38\ 88$	25	0 97
Girls	$AFM=54\ 33\times H^3-71\ 28$	17	0 98
$FEV_{1.0}$ to AFM			
Boys	$FEV_{1.0}=AFM\times 0\ 01308+0\ 8418$	0 15	0 99
Girls	$FEV_{1.0}=AFM\times 0\ 01272+0\ 8768$	0 13	0 99

sion lines of the AFM with respect to the body heights of the 73 healthy children The high correlation of the AFM units to the $FEV_{1.0}$ on the 73 healthy children is evident from Table 4 and Fig 1

Changes of VC, $FEV_{1.0}$ and AFM units on healthy children after inhalation of salbutamol are shown in Table 5 The increase of mean VC was not significant whereas the increases of mean $FEV_{1.0}$ and the mean AFM units are systematic For the two first groups of body heights 116–130 cm and 131–145 cm the changes of the AFM units are very similar and less than the change for the body heights 146–175 cm

DISCUSSION

According to the manufacturer the results should be the same whether the Monaghan spirometer is used with or without a backflow valve Our results show that this is true of $FEV_{1.0}$ but not of VC A mean difference of 0 14 l (Table 1) implies 4% of the mean VC value This systematic difference may depend upon the fact that some children begin to inhale before the removal of the mouthpiece after the FVC maneuver If the backflow valve is not used the inhalation causes a change of temperature recorded by the thermistor of the spirometer which give erroneously high VC values A substantial delay in the removal of the mouthpiece is probably observed by the investigator whereas a small delay can be overlocked This implies that the values obtained with the spirometer equipped with a valve should be the more correct ones For this reason we prefer to use the spirometer equipped with a valve

Our new reference data for $FEV_{1.0}$ of the Monaghan spirometer equipped with a back flow valve should thus be very similar to the data obtained in the investigation one year ago which is also the case (Table 2) The very close agreement of the $FEV_{1.0}$ values does credit to the spirometer The differences

Table 5 *Paired differences of vital capacity (VC) forced expiratory volume in one second ($FEV_{1.0}$) and Airflometer values (AFM) before and after inhalation of salbutamol (0 15 mg per kg body weight)*

For VC and $FEV_{1.0}$ the differences are expressed in percentages of the values before inhalation and within brackets in absolute values (l) For AFM the differences are given in absolute values i e arbitrary units and the results are divided in four groups according to body height

Lung function	M	S D	M+2 S D
VC			
Boys	+1 1 (0 03)	2 2 (0 08)	5 6
Girls	+0 2 (0 01)	1 6 (0 06)	3 4
$FEV_{1.0}$			
Boys	+3 6 (0 09)	2 3 (0 08)	8 1
Girls	+3 5 (0 07)**	2 7 (0 07)	8 9
AFM body height (cm)			
116–130	+5 8**	4 6	15 0
131–145	+4 8*	5 0	14 8
146–160	+9 6 *	5 9	21 4
161–175	+8 9 *	5 7	20 3

– =not significant =p<0 01

of VC probably depend upon the backflow valve in the way discussed above Other investigations of healthy Swedish children with water spirometers have however shown larger differences in results with one and the same type of spirometer i e water spirometers than those obtained by us on two different occasions with the Monaghan spirometer (2 3 5 7)

In our study a very high correlation (r= 0 99) was found between $FEV_{1\,0}$ of the Monaghan spirometer and the AFM units which agrees with findings on children and adults (1 6 11) Previous phantom studies have also disclosed good precision in this simple instrument (1 8) The correlation to $FEV_{1\,0}$ for the AFM should be better than for the Wright peak flow meter because not only the flow rate but also the time of flow influences the result To get measurable values of the AFM the subject must be capable of an exhalation corresponding to a $FEV_{1\,0}$ of about one litre which is a drawback when investigating small children All of our children did however cooperate very satisfactory in the AFM maneuver and all of them gave measurable results The correlation of AFM to body height is as high as that of $FEV_{1\,0}$ whereas the S D values around the lines are larger than those of $FEV_{1\,0}$ which agrees with previous findings on adults (1) Previous studies have shown a high reproducibility for one and the same AFM whereas the variations sometimes are considerable among different apparatuses (1 8 11)

In proper spirometric investigations of subjects with bronchial diseases the tests should also be done after administration of a bronchodilating drug In Sweden inhalations of salbutamol solution with compressor nebulizers are often used as the first treatment of acute asthma and also in testing the bronchial reaction for diagnostic purposes The bronchial response of healthy children was not previously evaluated but it has often been said that an increase of $FEV_{1\,0}$ of about 10% can be considered normal Souhrada & Buckley (9) state that significance is established only when the change in the spirometer values is 20% or greater In the study by Svenonius et al (10) 10 healthy children were investigated after exercise and inhalations of salbutamol There were no changes of mean VC or mean $FEV_{1\,0}$ In our study of 62 healthy children there was no systematic change in mean VC after inhalation of salbutamol (Table 5) Furthermore the deviations of the VC differences are small and not greater than those found in an ordinary duplicate investigation with a water spirometer (4 5) There was however a systematic change of $FEV_{1\,0}$ Despite the disturbing intermediate moment of inhalation the deviations of the $FEV_{1\,0}$ differences were not greater than the deviations found in an ordinary duplicate investigation with a water spirometer (5)

We have chosen a 6% increase of VC and 10% increase of $FEV_{1\,0}$ after administrations of salbutamol as normal upper limits which correspond to a value just above $M+2$ S D (Table 5)

Calculation of the change after inhalation of salbutamol in per cent is not possible for the AFM and the upper normal limits of change must be given in absolute AFM units An analysis of the change in relation to body height and original AFM values (Table 5) resulted in two limits an increase of 15 AFM units for the heights 116–145 cm and 21 AFM units for the heights 146–175 cm

ACKNOWLEDGEMENT

This investigation was supported by grants from Forenade Liv Mutual Life Insurance Company Stockholm and from Glaxo Lakemedel AB Goteborg Sweden

REFERENCES

1 Arborelius M & Christensson P Airflometer—en enkel apparat för lungfunktionstest *Lakartidn 75* 38 1978
2 Bjure J Spirometric studies in normal subjects IV Ventilatory capacities in healthy children 7–17 years of age *Acta Paediatr Scand 52* 23? 1963
3 Dalen G & Kjellman B Evaluation of three spirometers on healthy children *Acta Paediatr Scand* in press

Table 4 *The relations of the Airflometer values (AFM) to body height (H) and forced expiratory volume in one second ($FEV_{1 0}$) to AFM in healthy children*

The equations of linear regression the standard deviations (S D) and the coefficients of correlation (*r*) are given AFM is expressed in arbitrary units $FEV_{1 0}$ in litres and H in metres

Regression	Equation	S D	*r*
AFM to H			
Boys	$AFM = 45\ 78 \times H^2 - 38\ 88$	25	0 97
Girls	$AFM = 54\ 33 \times H^2 - 71\ 28$	17	0 98
$FEV_{1 0}$ to AFM			
Boys	$FEV_{1 0} = AFM \times 0\ 01308 + 0\ 8418$	0 15	0 99
Girls	$FEV_{1 0} = AFM \times 0\ 01272 + 0\ 8768$	0 13	0 99

sion lines of the AFM with respect to the body heights of the 73 healthy children The high correlation of the AFM units to the $FEV_{1 0}$ on the 73 healthy children is evident from Table 4 and Fig 1

Changes of VC $FEV_{1 0}$ and AFM units on healthy children after inhalation of salbutamol are shown in Table 5 The increase of mean VC was not significant whereas the increases of mean $FEV_{1 0}$ and the mean AFM units are systematic For the two first groups of body heights 116–130 cm and 131–145 cm the changes of the AFM units are very similar and less than the change for the body heights 146–175 cm

DISCUSSION

According to the manufacturer the results should be the same whether the Monaghan spirometer is used with or without a backflow valve Our results show that this is true of $FEV_{1 0}$ but not of VC A mean difference of 0 14 l (Table 1) implies 4% of the mean VC value This systematic difference may depend upon the fact that some children begin to inhale before the removal of the mouthpiece after the FVC maneuver If the backflow valve is not used the inhalation causes a change of temperature recorded by the thermistor of the spirometer, which give erroneously high VC values A substantial delay in the removal of the mouthpiece is probably observed by the investigator whereas a small delay can be overlocked This implies that the values obtained with the spirometer equipped with a valve should be the more correct ones For this reason we prefer to use the spirometer equipped with a valve

Our new reference data for $FEV_{1 0}$ of the Monaghan spirometer equipped with a backflow valve should thus be very similar to the data obtained in the investigation one year ago which is also the case (Table 2) The very close agreement of the $FEV_{1 0}$ values does credit to the spirometer The differences

Table 5 *Paired differences of vital capacity (VC) forced expiratory volume in one second ($FEV_{1 0}$) and Airflometer values (AFM) before and after inhalation of salbutamol (0 15 mg per kg body weight)*

For VC and $FEV_{1 0}$ the differences are expressed in percentages of the values before inhalation and within brackets in absolute values (l) For AFM the differences are given in absolute values i e arbitrary units and the results are divided in four groups according to body height

Lung function	*M*	S D	*M*+2 S D
VC			
Boys	+1 1 (0 03)	2 2 (0 08)	5 6
Girls	+0 2 (0 01)	1 6 (0 06)	3 4
$FEV_{1 0}$			
Boys	+3 6 (0 09)*	2 3 (0 08)	8 1
Girls	+3 5 (0 07)**	2 7 (0 07)	8 9
AFM body height (cm)			
116–130	+5 8*	4 6	15 0
131–145	+4 8**	5 0	14 8
146–160	+9 6**	5 9	21 4
161–175	+8 9*	5 7	20 3

* =not significant ** $=p<0\ 01$

Acta Pædiatr Scand 68 109–111 1979

REDUCED SERUM 1 25 (OH) VITAMIN D_3 LEVELS IN PREDNISONE TREATED ADOLESCENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

SEAN O REGAN R W CHESNEY A HAMSTRA J A EISMAN
A M O GORMAN and H F DELUCA

From the Departments of Nephrology and Radiology McGill University Montreal Children s Hospital Montreal Canada Research Institute Montreal Canada and the Departments of Pediatrics and Biochemistry University of Wisconsin Madison Wisc USA

ABSTRACT O Regan S Chesney R W Hamstra A Eisman J A O Gorman A M and DeLuca H F (Departments of Nephrology and Radiology McGill University Montreal Children s Hospital Montreal Canada Research Institute Montreal Canada and the Departments of Pediatrics and Biochemistry University of Wisconsin Madison Wisc USA) Reduced serum 1 25-$(OH)_2$ vitamin D levels in prednisone treated adolescents with systemic lupus erythematosus Acta Paediatr Scand 68 109 1979 —The serum levels of 1,25-$(OH)_2$ vitamin D_3 were assayed in samples from 12 adolescent patients with SLE Subnormal levels were observed in 7 of these 12 patients Low levels of the metabolically active polar metabolite of vitamin D may contribute to the development of osteopenia observed in this disease The cumulative effects of the osteoporotic and anti vitamin D effects of long term steroid therapy in children with SLE may require the cautious administration of supplemental vitamin D

KEY WORDS 1 25 $(OH)_2$ D steroids SLE

Recent studies suggest that avascular necrosis of bone may be a common complication of systemic lupus erythematosis (SLE) (1 7) These bone lesions usually adjacent to joint surfaces may cause severe pain or incapacitating structural deformities Although steroids have been considered to be a contributing factor the diffuse vasculitis of SLE has also been suggested as a possible cause (1) particularly since osteonecrosis has also occurred in the absence of steroid therapy (3 10) Thus steroid therapy has obscured the distinction between the role of the disease itself and that of the treatment in the pathogenesis of bone disease in SLE

To determine if derangements in vitamin D_3 metabolism a major factor in normal bone deposition and reabsorption might exist and thus possibly contribute to the development of bone lesions in SLE we have assayed the serum levels of 1 25 $(OH)_2$ D_3 a major active polar metabolite of vitamin D_3 in 12 children with SLE of long duration and thus at risk for developing bone lesions This study also afforded the opportunity to determine if chronic steroid therapy might be associated with altered 1 25 (OH) vitamin D_3 metabolism as previous reports have indicated that both normal (6) and decreased (8) serum 25 (OH) D_3 levels may be associated with chronic steroid ingestion

MATERIALS AND METHODS

Serum samples of 12 children with SLE were assayed for 1,25-(OH) D_3 The diagnosis of SLE was confirmed by the presence of laboratory positive LE cell preparations elevated antinuclear antibody (ANA) titers low serum C levels and clinical features consistent with the diagnosis All had lupus nephritis confirmed by light electron and immunofluorescence microscope examination of renal biopsy material All had been or were undergoing treatment with a combination of prednisone and azathioprine

All available radiologic examinations were re-examined

4 Engström I Karlberg P & Kriepelien S Respiratory studies in children I Lung volumes in healthy children 6–14 years of age *Acta Paediatr Scand 46* 277 1956
5 Engström I Karlberg P & Swarts C L Respiratory studies in children IX Relationships between mechanical properties of the lungs lung volumes and ventilatory capacity in healthy children 7–15 year of age *Acta Paediatr Scand 51* 68 1962
6 Friedman M & Walker S Assessment of lung function using an air flowmeter *Lancet 1* 310 1975
7 Kjellman B Ventilatory efficiency capacity and lung volumes in healthy children *Scand J Clin Lab Invest* 23 19 1969
8 Ringqvist I Sixt R Bake B & Oxhoj H Validity of the Airflometer A simple device for assessment of forced expiration *Scand J Resp Dis 58* 265 1977
9 Souhrada J F & Buckley J M Pulmonary function testing in asthmatic children *Ped Clin North Amer 23* 249 1976
10 Svenonius E Lecerof H Lilja B & Arborelius M Jr The volume of trapped gas A new and sensitive test for the detection of exercise induced bronchospasm in children *Acta Paediatr Scand* in press
11 Thompson P Friedman M & Walker S The Airflometer—A new device for assessing changes in respiratory function *Practitioner 219* 3 1977

Submitted April 15 1978
Accepted June 2 1978

(G D) Department of Paediatrics
Karnsjukhuset
S 541 01 Skovde
Sweden

min D_3 therapy at 0 4 μg per day restored normal intestinal calcium transport (5) It appeared that daily prednisone therapy was necessary to produce decreased 25 (OH) vitamin D_3 levels though Hahn et al (6) have recently reported normal serum levels of 25 (OH) D_3 levels in patients on chronic corticosteroid therapy However in our adolescent patients prednisone therapy was associated with reduced 1 25 $(OH)_2$ vitamin D_3 levels Though serum urea nitrogen and creatinine levels were normal this does not rule out the possibility that tubular function was inadequate for 1α Hydroxylation although as mentioned above steroid therapy may alter 1α 25 (OH) D_3 metabolism (2) Dietary availability of vitamin D was >250 units/daily in all these patients hence inadequate intake was unlikely Florid rachitic changes were not evident radiologically in any of the patients Low calcium levels were evident in only two cases and these patients also had low 1–25 (OH) D_3 levels

The observation that 7 of 12 children with SLE had low levels of 1 25 (OH) D_3 possibly related to chronic steroid therapy of vitamin D_3 suggests that routine dietary intake may be inadequate for these patients The cumulative effects of the osteoporotic and anti vitamin D effects of long term steroid therapy in children with SLE may require the cautious administration of supplemental vitamin D as has been used in other patients with exogenous hypercortisonism (5 8)

REFERENCES

1 Bergstein J M Wiens C Fish A J Vernier R L & Michael A Avascular necrosis of bone in systemic lupus erythematosis *J Pediatr 85* 31 1974

2 Carre M Ayigbede P Miravet L & Rasmussen H The effect of prednisone upon the metabolism and action of 25 Hydroxy and 1 25 D hydroxy vitamin D_3 *Proc Natl Acad Sci 71* 2996 1974

3 Dubois E L & Cozen L Avascular (aseptic) bone necrosis associated with systemic lupus erythematosus *JAMA 174* 966 1960

4 Eisman J A Haustra A J Kream B E & DeLuca H F 1 25 D Hydroxy vitamin D in biological fluids a simplified and sensitive assay *Science 193* 1021 1976

5 Hahn T J & Hahn B H Osteopenia in patients with rheumatic diseases principles of diagnosis and therapy *Semin Arthritis Rheum 6* 165 1976

6 Hahn T J Halstead L R & Haddad J G Serum 25 Hydroxy vitamin D concentration in patients receiving chronic corticosteroid therapy *J Lab Clin Med 90* 399 1977

7 Hurley R M Steinberg R M Patriquin H & Drummond K N Avascular necrosis of the femoral head in childhood systemic lupus erythematosus *Can Med Assoc J 111* 79 1974

8 Klein R G Arnaud S B Gallagher J C DeLuca H F & Riggs B L Intestinal calcium absorption in exogenous hypercortisonism Role of 25 hydroxy vitamin D and corticosteroid dose *J Clin Invest 60* 253 1977

9 Lukert B P Stanbury S W & Mawer E B Vitamin D and intestinal transport of calcium effects of prednisone *Endocrinology 93* 718 1973

10 Siemsen J K Brook J & Meister L Lupus erythematosus and avascular bone necrosis a clinical study of three cases and review of the literature *Arthritis Rheum 5* 492 1962

11 Sutton R D Aseptic necrosis of bone a complication of corticosteroid therapy *Drug Ind Dis 3* 171 1968

Submitted March 9 1978
Accepted June 25 1978

(S O R) Department of Nephrology
Hopital Sainte Justine
3175 Chemin Cote Ste Catherine
Montreal
Quebec H3T 1C5
Canada

Table 1 *Serum 1 25 $(OH)_2$ D_3 levels in SLE patients*

Patient no	Duration of disease (years)	Radiologic assessment	Serum Ca Phos Alk Phos	Serum 1 25 (OH) D_3 pg/ml (normal 40 8±2 6 pg/ml)
1	1	Mild osteopenia	Normal	12
				17
2	5	Normal	Normal	30
				115 (on vit D_3)
3	7	Mild osteopenia	Normal	15
4	8	Mild osteopenia	Normal	32
5	4	Mild osteopenia	Normal	25
6	4	Mild osteopenia	Serum Ca↓	11
				62 (on vit D_3)
7	5	Normal	Normal	59
8	1	Mild osteopenia	Normal	82
9	1	Mild osteopenia	Normal	48
10	2	Mild osteopenia	Normal	0
11	5	Normal	Normal	42
12	3	Mild osteopenia	Serum Ca↓	12

for evidence of bone lesions The serum C_3 level ANA and parameters of renal function were also assessed Serum Ca phosphate and alkaline phosphatase levels were also measured using a Technicon autoanalyzer 1 25-$(OH)_2$ D_3 levels were assayed by a modification of the method of Eisman et al (4) This assay is specific for 1 25 $(OH)_2$ D_3 Values obtained were compared with values obtained in 56 normal children of ages 8–18 years

RESULTS

Duration of disease in these patients varied from one to eight years patient ages varied from 12–19 years There were 8 females and 4 males Normal values for 1 25 $(OH)_2$ vitamin D_3 in healthy children ages 8–18 was 40 8±2 6 pg/ml (mean±1 S E) (range 19–111 pg/ml) Seven of the 12 patients had subnormal levels of 1 25 $(OH)_2$ D_3 (Table 1) On later assay 2 of the 7 had normal levels

All patients had normal serum creatinine and BUN concentrations Liver function studies (SGOT SGPT LDH) available on 6 patients were normal Eleven patients had received or were receiving prednisone 50–80 mg every other day and azathioprine therapy 50 mg daily One patient was receiving prednisone 60 mg/day Serum calcium phosphate and alkaline phosphatase were normal in all but 2 patients Radiographic studies available in all patients did not demonstrate any major bone lesions or rachitic changes though osteopenia was prominent on many of the examinations Dietary history revealed an intake of >250 units vitamin D_3 per day in each patient Patients are told to avoid sunlight and to use various sunscreen agents when exposure was anticipated

DISCUSSION

Previous studies of children and adults with SLE indicate this group of patients is at risk for developing bone lesions Corticosteroid administration in SLE and other diseases has been associated with osteonecrosis (11) and osteoporosis This may in part be related to the inhibition of the intestinal transport of calcium by steroids (9) Steroids have been demonstrated to alter 1 25 (OH) D_3 metabolism in the rat leading to the formation of more polar biologically inactive metabolites that may result in decreased intestinal calcium transport (2) Recently it has been demonstrated that serum 25 (OH) vitamin D_3 levels are reduced in corticosteroid treated adult patients with a variety of collagen vascular diseases and rheumatoid arthritis (8) The reduction in gut calcium transport in these patients appeared to be related to the lower serum 25 (OH) vitamin D_3 levels and treatment with 1 25 $(OH)_2$ vita

min D_3 therapy at 0 4 μg per day restored normal intestinal calcium transport (5) It appeared that daily prednisone therapy was necessary to produce decreased 25 (OH) vitamin D_3 levels though Hahn et al (6) have recently reported normal serum levels of 25 (OH) D_3 levels in patients on chronic corticosteroid therapy However in our adolescent patients prednisone therapy was associated with reduced 1 25 $(OH)_2$ vitamin D_3 levels Though serum urea nitrogen and creatinine levels were normal this does not rule out the possibility that tubular function was inadequate for 1α Hydroxylation although as mentioned above steroid therapy may alter 1α 25 (OH)-D_3 metabolism (2) Dietary availability of vitamin D was >250 units/daily in all these patients hence inadequate intake was unlikely Florid rachitic changes were not evident radiologically in any of the patients Low calcium levels were evident in only two cases and these patients also had low 1–25 (OH) D_3 levels

The observation that 7 of 12 children with SLE had low levels of 1 25 $(OH)_2$ D_3 possibly related to chronic steroid therapy of vitamin D_3 suggests that routine dietary intake may be inadequate for these patients The cumulative effects of the osteoporotic and anti vitamin D effects of long term steroid therapy in children with SLE may require the cautious administration of supplemental vitamin D as has been used in other patients with exogenous hypercortisonism (5 8)

REFERENCES

1 Bergstein J M Wiens C Fish A J Vernier R L & Michael A Avascular necrosis of bone in systemic lupus erythematosis *J Pediatr 85* 31 1974

2 Carre M Ayigbede P Miravet L & Rasmussen H The effect of prednisone upon the metabolism and action of 25 Hydroxy and 1 25 D hydroxy vitamin D_3 *Proc Natl Acad Sci 71* 2996 1974

3 Dubois E L & Cozen L Avascular (aseptic) bone necrosis associated with systemic lupus erythematosus *JAMA 174* 966 1960

4 Eisman J A Haustra A J Kream B E & DeLuca H F 1 25 D Hydroxy vitamin D in biological fluids a simplified and sensitive assay *Science 193* 1021 1976

5 Hahn T J & Hahn B H Osteopenia in patients with rheumatic diseases principles of diagnosis and therapy *Semin Arthritis Rheum 6* 165 1976

6 Hahn T J Halstead L R & Haddad J G Serum 25 Hydroxy vitamin D concentration in patients receiving chronic corticosteroid therapy *J Lab Clin Med 90* 399 1977

7 Hurley R M Steinberg R M Patriquin H & Drummond K N Avascular necrosis of the femoral head in childhood systemic lupus erythematosus *Can Med Assoc J 111* 79 1974

8 Klein R G Arnaud S B Gallagher J C DeLuca H F & Riggs B L Intestinal calcium absorption in exogenous hypercortisonism Role of 25 hydroxy vitamin D and corticosteroid dose *J Clin Invest 60* 253 1977

9 Lukert B P Stanbury S W & Mawer E B Vitamin D and intestinal transport of calcium effects of prednisone *Endocrinology 93* 718 1973

10 Siemsen J K Brook J & Meister L Lupus erythematosus and avascular bone necrosis a clinical study of three cases and review of the literature *Arthritis Rheum 5* 49? 196?

11 Sutton R D Aseptic necrosis of bone a complication of corticosteroid therapy *Drug Ind Dis 3* 171 1968

Submitted March 9 1978
Accepted June 25 1978

(S O R) Department of Nephrology
Hopital Sainte Justine
3175 Chemin Cote Ste Catherine
Montreal
Quebec H3T 1C5
Canada

Acta Pædiatr Scand 68 113–118 1979

EFFECT OF INTRAVENOUS HYDROCORTISONE ADMINISTRATION ON GLUCOSE HOMEOSTASIS IN SMALL FOR GESTATIONAL AGE INFANTS

L SANN A RUITTON M MATHIEU and Y LASNE

From the Department of Neonatology Unite INSERM U34 and Laboratoire d'Enzymologie Hopital Debrousse Lyon France

ABSTRACT Sann L Ruitton A Mathieu M and Lasne Y (Department of Neonatology INSERM U34 and Laboratory of Enzymology Hospital Debrousse Lyon France) Effect of intravenous hydrocortisone administration in small for gestational age infants Acta Paediatr Scand 68 113 1979.—The effects of I V hydrocortisone (H) (10 mg/kg) on glucose homeostasis were evaluated at 25 to 85 hours of age in 14 infants who were small for gestational age (SGA) in comparison to 17 control SGA infants Three hours after H administration higher levels of plasma glucose than in controls were detected (mean ± S E M) 4 78±0 2 vs 2 88+0 2 mmol/l ($p<0\ 01$) while lower levels were found for blood pyruvate (38±7 vs 89±12 μmol/l—$p<0\ 01$) plasma insulin (6 4±0 5 vs 12±0 8 μIU/ml—$p<0\ 05$) and plasma glucagon (62 25±6 6 vs 81 6±6 6 pmol/l—$p<0\ 05$) Three hours after H administration I V injection of L alanine (150 mg/kg) produced a significant rise over baseline of plasma glucose concentration from 4 78±0 2 to 5 94±0 2 mmol/l at 50 min ($p<0\ 05$) whereas no significant change was observed in controls There was no significant change in plasma glucagon and insulin concentrations after L alanine injection in either group These results show that in SGA infants primed with H the rise of plasma glucose concentration after L alanine administration is observed with low plasma insulin levels and without stimulation of glucagon secretion They suggest that H induced a *reduced* peripheral utilization of glucose by lowering the plasma levels of insulin and a *production* of glucose from alanine through gluconeogenesis

KEY WORDS Infants glucose homeostasis hydrocortisone plasma insulin alanine gluconeogenesis

Small for gestational age (SGA) infants are at risk from hypoglycemia (2 12) This predisposition to hypoglycemia is attributed to decreased hepatic glycogen stores (3 24) and/or inadequate gluconeogenesis (11) A major treatment for neonatal hypoglycemia is provided by glucocorticoids like hydrocortisone (H) (2 24) Hyperglycemia induced by corticoids is due to reduced peripheral utilization of glucose (17) increased secretion and effect of glucagon (27) and enhanced gluconeogenesis (5 23) In newborn infants H has been shown to reduce peripheral glucose utilization (9) and to increase the effect of exogenous glucagon (18) But the other mechanisms have not been investigated

The present work was designed to study in SGA infants the effects of H administration on the glycemic response to L alanine in SGA infants

MATERIAL AND METHODS

Subjects Thirty-one newborn infants were studied during the first three days of life (Table I) All study procedures were approved by the ethic committee of the Department of Neonatology and of the Unite de Recherche U34 Hopital Debrousse All infants were SGA their birthweight being under the 10th percentile of the intrauterine growth curve (13) Their gestational age was determined by menstrual history and by clinical assessment according to the method of Dubowitz et al (13) Infants with Apgar scores lower than five and infants whose mothers had previously received betamethasone injections were excluded *Feeding* was started at the age of six hours with 40 to 60 ml/kg/24 h the first day 60 to 80 ml/kg/24 h the second and 80 to 100 ml/kg/24 h

Table 1 *Main clinical data*

Small for date infants	No	Gestational age (weeks)	Birth weight (g)	Postnatal age (hours)	Complications
Without hydrocortisone	17	38 3 (35–42)	1 890 (1 590–2 250)	41 (25–82)	Toxemia=4 Twins=?
With hydrocortisone	14	37 8 (35–42)	2 000 (1 640–2 360)	43 8 (34–56)	Toxemia=5 Twins=2 Cesarean section=1

Mean ranges in parentheses

the third day with a commercial milk providing 67 cal/100 ml and lactose 7 25 g/100 ml

In addition all infants were infused at entrance into the unit with 10% glucose in order to achieve with feeding a daily input of 10 g/kg glucose The infants were separated into two groups group I 17 control SGA infants and group II 14 SGA infants with hydrocortisone administration The main clinical data of these groups are shown in Table 1 Five infants developed hypoglycemia (i e plasma glucose levels lower than 1 65 mmol/l) in group I and four in group II At the end of the glucose infusion plasma glucose levels were similar in both groups (mean and ranges) in group I 5 06 mmol/l (1 81–10) and in group II 5 33 mmol/l (2 25–9 07)

Procedures Feeding and glucose infusion were discontinued three–four hours prior to the test and isotonic saline solution was infused at the rate of 0 5 ml/min In group II hydrocortisone hemisuccinate (10 mg/kg) was injected intravenously three hours before alanine administration L Alanine in a sterile solution prepared in the laboratory of the hospital was administrated intravenously at the dose of 150 mg/kg of body weight into a scalp vein over a period of 30 to 60 sec Blood samples were collected through an arterial umbilical catheter previously inserted at 0 10 20 30 and 40 min only blood glucose pyruvate and lactate were studied at 50 min to reduce the number of blood samples

Measurements 0 1 ml of blood was collected for glucose determination by a glucose-oxidase method (Beckman glucose Analyser) Specimens of 0 5 ml collected in heparinized microtubes were immediately deproteinized by perchloric acid (0 6 M) and blood pyruvate B hydroxybutyrate and lactate were determined immediately by an enzymatic method (25 27) Blood samples were collected in heparinized tubes and in tubes with EDTA and trasylol for insulin and glucagon determinations (1 5 ml) The tubes were kept in ice and immediately centrifuged at 4 C Plasma was removed and frozen until assayed The samples were analysed for insulin by the method of Hales & Randle (10) and for glucagon by a radio immunoassay which did not cross with purified gut glucagon (11) The results were analysed by means of the Fisher test followed by the Student s and paired-t test

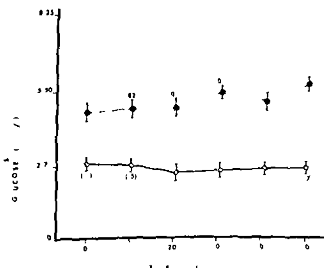


Fig 1 Effect of L alanine (150 mg/kg) on plasma glucose concentration (mean ± S E M) in control SGA infants (O—O) and in group II three hours after hydrocortisone (H) administration● ● – the number of infants is given in parentheses * significant level over baseline ($p<0\ 05$) (paired t test)

RESULTS

Base line levels Plasma glucose concentration was higher in group II than in the control group I (mean ± S E M) 4 78±0 2 mmol/l vs 2 88±0 2 mmol/l ($p<0\ 01$) Plasma concentrations of glucagon and insulin were lower after H administration 62 25±6 6 vs 81±6 6 pmol/l in group I ($p<0\ 05$) and 6 42±0 5 vs 12±0 1 µUI/ml in group I ($p<0\ 05$) respectively But the insulin/glucagon molar ratio was not significantly different in group I (1 09±0 13) and group II (0 82±0 08) ($p>0\ 05$) The basal level of blood pyruvate was lower after H administration 38 42±7 52 µmol/l than in group I 89 73±11 81 µmol/l No significant differ

Table 2 *Effect of L alanine (150 mg/kg) on plasma glucagon and insulin concentrations and insulin/glucagon molar ratio in control (group I) and hydrocortisone primed (group II) infants*

	Time (min) 0	10	20	30	40
Plasma glucagon (pmol/l)					
Group I	8 ±6 6 (17)	98±10 (14)	113±10 (15)	11[illegible]±12 (16)	106±13 (16)
Group II	6[illegible]±6 6 (14)	56±5 7 (11)	69±7 5 (14)	63±5 7 (14)	6[illegible]±6 0 (14)
Plasma insulin (μU/ml)					
Group I	1[illegible]±1 (17)	15±[illegible] (14)	14±1 (16)	12±1 (17)	11±1 (17)
Group II	6 4±0 5 (14)	6 9±0 7 (11)	8 0±1 (14)	7 9±1 (11)	7 4±1 (14)
Insulin/glucagon (molar ratio)					
Group I	1 09±0 13 (17)	1 19±0 18 (14)	0 96±0 1[illegible] (14)	0 84±0 1[illegible] (16)	0 82±0 1[illegible] (16)
Group II	0 8[illegible]±0 08 (14)	0 9[illegible]±0 08 (11)	0 86±0 08 (14)	0 95±0 09 (11)	0 90±0 08 (14)

Mean ± S E M number in parentheses=number of infants

ence was observed with the B hydroxybutyrate levels 79 2±12 μmol/l in group I and 118±23 μmol/l in group II ($p>0$ 05)

Response to L alanine administration The effect of L alanine infusion on the mean glucose concentration is shown in Fig 1 A significant rise over the baseline level from 4 78±0 2 to 5 94±0 2 mmol/l ($p<0$ 01) was observed after H administration while no significant change (from 2 88±0 2 to 2 59±0 2 mmol/l) was found in the control group Intravenous alanine administration produced no significant change in plasma glucagon plasma insulin concentrations and insulin/glucagon molar ratio (Table 2) or blood lactate and pyruvate concentrations (Table 3) in either group

DISCUSSION

In a previous study performed exactly under the same conditions (22) we observed that in normal full term infants the intravenous administration of L alanine produced a significant rise of plasma glucose concentrations over the baseline levels in premature infants it induced a small but significant decrease in plasma glucose concentration which subsequently was found at the baseline level These effects were observed with an increase in plasma glucagon concentrations but no change of plasma insulin levels in both groups In the SGA infants of the control group none of these effects were observed since there was no change of plasma glucose glucagon

Table 3 *Effect of L alanine (150 mg/kg) on blood pyruvate and lactate concentrations in control (group I) and hydrocortisone primed (group II) infants*

Time (min)	Pyruvate (μmol/l) Group I	Pyruvate (μmol/l) Group II	Lactate (μmol/l) Group I	Lactate (μmol/l) Group II
0	89±1[illegible]	38± 7	9 7±97[illegible]	3 [illegible]83± 594
10	90±13	31± 7	3 140±840	3 484± 430
20	91±1	45± 7	3 030±9 0	3 574± 830
30	89±1[illegible]	3 ± 8	6 0±5 0	[illegible] 808± 830
40	88±14	46± 9	[illegible] 360±450	3 3[illegible]± 860
50	87±16	45±10	1 860±340	3 473±1 116

Mean ± S E M

Table 1 *Main clinical data*

Small for date infants	No	Gestational age (weeks)	Birth weight (g)	Postnatal age (hours)	Complications
Without hydro-cortisone	17	38 3 (35–42)	1 880 (1 590–2 250)	41 (25–82)	Toxemia=4 Twins=2
With hydro cortisone	14	37 8 (35–42)	2 000 (1 640–2 360)	43 8 (34–56)	Toxemia=5 Twins=2 Cesarean section=1

Mean ranges in parentheses

the third day with a commercial milk providing 67 cal/100 ml and lactose 7 25 g/100 ml

In addition all infants were infused at entrance into the unit with 10% glucose in order to achieve with feeding a daily input of 10 g/kg glucose The infants were separated into two groups group I 17 control SGA infants and group II 14 SGA infants with hydrocortisone administration The main clinical data of these groups are shown in Table 1 Five infants developed hypoglycemia (i e plasma glucose levels lower than 1 65 mmol/l) in group I and four in group II At the end of the glucose infusion plasma glucose levels were similar in both groups (mean and ranges) in group I 5 06 mmol/l (1 81–10) and in group II 5 33 mmol/l (2 25–9 07)

Procedures Feeding and glucose infusion were discontinued three-four hours prior to the test and isotonic saline solution was infused at the rate of 0 5 ml/min In group II hydrocortisone hemisuccinate (10 mg/kg) was injected intravenously three hours before alanine administration L Alanine in a sterile solution prepared in the laboratory of the hospital was administrated travenously at the dose of 150 mg/kg of body w into a scalp vein over a period of 30 to 60 sec B samples were collected through an arterial umb catheter previously inserted at 0 10 20 30 and 40 only blood glucose pyruvate and lactate were st at 50 min to reduce the number of blood samples

Measurements 0 1 ml of blood was collected for cose determination by a glucose-oxidase method (B man glucose Analyser) Specimens of 0 5 ml coll in heparinized microtubes were immediately depro ized by perchloric acid (0 6 M) and blood pyruvat hydroxybutyrate and lactate were determined i diately by an enzymatic method (25 27) Blood sa were collected in heparinized tubes and in tubes EDTA and trasylol for insulin and glucagon deter tions (1 5 ml) The tubes were kept in ice and immed centrifuged at 4 C Plasma was removed and frozen assayed The samples were analysed for insulin b method of Hales & Randle (10) and for glucagon radio immunoassay which did not cross with purifie glucagon (11) The results were analysed by means c Fisher test followed by the Student s and paired-t tes

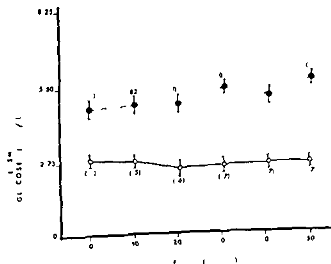


Fig 1 Effect of L alanine (150 mg/kg) on plasma glucose concentration (mean ± S E M) in control SGA infants (O—O) and in group II three hours after hydrocortisone (H) administration● ● – the number of infants is given in parentheses * significant level over baseline ($p<0.05$) (paired t test)

RESULTS

Base line levels Plasma glucose concentra was higher in group II than in the cor group I (mean ± S E M) 4 78±0 2 mmol/ 2 88±0 2 mmol/l ($p<0.01$) Plasma concer tions of glucagon and insulin were lower a H administration 62 25±6 6 vs 81±6 6 pn in group I ($p<0.05$) and 6 42±0 5 vs 12 μUI/ml in group I ($p<0.05$) respectively the insulin/glucagon molar ratio was not nificantly different in group I (1 09±0 13) group II (0 82±0 08) ($p>0.05$) The basal le of blood pyruvate was lower after H ministration 38 42±7 52 μmol/l than in gr I 89 73±11 81 μmol/l No significant dif

Langerhans may inhibit insulin secretion Such an effect has been demonstrated by Colle & Goldman (1) by in vitro studies on pancreas of human fetuses and premature infants In addition they showed that H has no effect on glucagon release

In conclusion this study shows that after administration of pharmacological doses of hydrocortisone an hyperglycemic response to L alanine is observed without glucagon stimulation in SGA The occurrence of this hyperglycemic response is probably facilitated by the reduction of peripheral utilization of glucose These results also suggest that the rise of plasma glucose concentration after alanine administration is due to direct conversion of alanine into glucose Lower plasma insulin concentrations after H administration may also explain these results

REFERENCES

1 Colle E & Goldman H Effect of glucocorticoids on insulin release from human fetal islets of Langerhans *Diabetes 25* Suppl 1 359 1976

Cornblath M & Schwartz R *Disorders of carbohydrate metabolism in infancy* W B Saunders Philadelphia 1966 p 4?

3 Cornblath M Wybregt S H Baens G S & Klein R I Symptomatic neonatal hypoglycemia studies of carbohydrate metabolism in the newborn infant VIII *Pediatrics 33* 388 1964

4 Dubowitz L M S Dubowitz R & Goldberg C Clinical assessment of gestational age in the new born infant *J Pediatr 77* 1 1970

5 Exton J H Gluconeogenesis *Metabolism 21* 945 197?

6 Felig P Pozefsky T Marliss E & Cahill G F Jr Alanine role in gluconeogenesis *Science 167* 1003 1970

7 Fernandez J & Bloom W The intravenous L alanine tolerance test as a mean for investigating gluconeogenesis *Metabolism 23* 1449 1974

8 Fiser R H Jr Williams P R Fisher D A Delamater P V Sperling M A & Oh W The effect of oral alanine on blood glucose and glucagon in the human newborn infant *Pediatrics 56* 78 1975

9 Gentz J C H Warner R Persson B E & Cornblath M Intravenous glucose tolerance plasma insulin free fatty acids and beta hydroxy butyrate in underweight newborn infants *Acta Paediatr Scand 58* 481 1969

10 Hales C N & Randle P J Immunoassay with insulin antibody precipitate *Biochem J 88* 137 1963

11 Haymond M W Karl I E & Pagliara A S Increased gluconeogenic substrates in the small for gestational age infant *N Engl J Med 291* 3?2 1974

1? Lubchenco L O & Bard H Incidence of hypoglycemia in newborn infants classified by birthweight and gestational age *Pediatrics 47* 831 1971

13 Lubchenco L O Hansmann C & Boyd E Intrauterine growth in length and head circumference as estimated from live births at gestational ages from 26 to 4? weeks *Pediatrics 37* 403 1966

14 Marco J Calle C Roman D Diaz Fierros M Villanueva M L & Valverde I Hyperglucagonism induced by glucocorticoid treatment in man *N Engl J Med 288* 1 8 1973

15 Martin R J Patt J A & Eberhatt R J The effects of metyrapone and ACTH on the development of gluconeogenesis in the neonatal pig *Proc Soc Exp Biol Med 144* 603 1973

16 Muller W A Faloara G R & Unger R H Jr Effect of alanine on glucagon secretion *J Clin Invest 50* ??15 1971

17 Munck A Glucocorticoid inhibition of glucose uptake by peripheral tissues old and new evidence molecular mechanisms and physiological evidence *Perspect Biol Med 14* 265 1971

18 Oh W Baens G S Migeon C J Wybregt S H & Cornblath M Studies of carbohydrates metabolism in the newborn infant V The effect of cortisol on the hyperglycemic response to glucagon *Pediatrics 30* 769 1962

19 Pagliara S A Karl I E De Vivo D C Feigin R D & Kipnis D M Hypoalaninemia a concomitant of ketotic hypoglycemia *J Clin Invest 51* 1440 197?

20 Price H V Courley T & Cameron E H D Neonatal hydrocortisone administration effect on cortisol and glucose levels *Biol Neonate 30* 138 1976

21 Ruitton A & Frederich F Dosage radioimmunologique spécifique du glucagon pancreatique Applications chez le nouveau ne et l enfant *Metabolisme et Diabete 1* 143 1975

2 Sann L Ruitton A Mathieu M Bourgeois J & Genoud J Effect of intravenous L alanine administration on plasma glucose insulin and glucagon blood pyruvate lactate and Beta hydroxybutyrate concentrations in newborn infants Study in appropriate term and preterm newborn infants *Acta Paediatr Scand 67* ?97 1978

3 Seitz H J Kaiser M Krone W & Tarnowski W Physiologic significance of glucocorticoids and insulin in the regulation of hepatic gluconeogenesis during starvation in rats *Metabolism 25* 1545 1976

?4 Shelley H J & Neligan G A Neonatal hypoglycemia *B Med Bull 22* 34 1966

5 Tfelt P & Sigaard Anderson O Lactate and pyruvate determinations in 50 µl whole blood *Scand J Lab Invest 27* 15 1971

6 Vildnes J & Sovik O Gluconeogenesis in infancy and childhood II Studies of the glucose production from alanine in three cases of persistant neonatal hypoglycemia *Acta Paediatr Scand 65* 307 1976

and insulin concentrations after alanine administration Our results in these SGA infants agree with the findings of Williams et al (28) with oral administration of alanine Blood lactate and pyruvate concentrations were measured in SGA control infants to find out whether a partial defect in hepatic gluconeogenesis could be detected (7) However there were no changes of blood lactate and pyruvate concentrations after L alanine administration These results do not suggest a defect of gluconeogenesis (7) although a defect in phosphoenol pyruvate carboxykinase cannot be completely ruled out (26)

In the control infants of group I the production of glucose after alanine administration could be balanced by a marked peripheral utilization of glucose this could explain that plasma glucose concentration did not change after alanine administration On the contrary in the SGA infants primed with hydrocortisone (group II) the basal levels of glucose were higher than in group I These results agree with the hyperglycemic effect of H As shown by Gentz et al (9) it could be explained by the reduction of peripheral glucose utilization This could be supported in our study by the depressed plasma insulin levels in group II However the rise of plasma glucose concentration after alanine administration also indicates that some glucose was produced from alanine

This study gives also some indications of the process of glucose production in these infants In adults and children the hyperglycemic response to alanine has been attributed to increased secretion of glucagon (16) and/or to direct conversion of alanine into glucose through gluconeogenesis (6 19) In the H primed SGA infants hyperglycemia was induced by L alanine despite lower levels of plasma glucagon than in the control group and without any change of plasma glucagon concentration or of insulin/glucagon molar ratio Therefore these results do not support a participation of glucagon to the hyperglycemic response to alanine in H primed SGA infants

These data are different from the findings o a study in adults where dexamethasone give for three days induced an increase in plasm glucose and glucagon concentration (14)

Since glycogenolysis was not stimulate through glucagon these data rather sugges that the rise of plasma glucose concentratio occurred through gluconeogenesis in thes infants This assumption is supported by th lower concentrations of blood pyruvate i group II (23) This is also supported by th absence of any change in serum insulin an glucagon concentrations after L alanine ad ministration Finally, it would be in agreemen with studies in neonatal pigs showing that I improves their gluconeogenic capacities (1⁵ A direct effect of H on gluconeogenesis coul be limited by the delay of enzyme activatio by H However H stimulates the activity c the gluconeogenic enzymes rather than thei synthesis (23) Therefore a direct influence c H on alanine transformation into glucos cannot be excluded in the infants of grou II However this conclusion could be demon strated in SGA infants only by quantitativ measurements which are difficult to realize i newborn infants for technical and ethical rea sons Further studies are required to deter mine the exact participation of H to these results

The absence of glucagon and insulin stimu lation by alanine in group II is similar to the findings in the control group The lower levels of plasma insulin and glucagon in group II were surprising results since in adults acute administration of H did not influence the plasma concentrations of insulin and glucagon (14) Hyperglycemia could have precluded the induction of a rise in plasma glucagon as shown by Fiser et al (8) However this effect could not explain the lower level of insulin in group II Impairment of cellular penetration of glucose by H (6) could account for the absence of insulin response to hyperglycemia but it could not be applied to the lower levels of plasma glucagon Finally a direct suppressive influence of H on the β cells of

CASE REPORT

A VARIANT OF THE KLIPPEL–TRENAUNAY–WEBER SYNDROME WITH TEMPORAL LOBE ASTROCYTOMA

P HOWITZ J HOWITZ and F GJERRIS

From the Departments of Paediatrics Dermatology and Neurosurgery University of Copenhagen Rigshospitalet Copenhagen Denmark

ABSTRACT Howitz P Howitz J and Gjerris F (Departmetns of Paediatrics Dermatology and Neurosurgery University of Copenhagen Rigshospitalet Copenhagen Denmark) A variant of the Klippel–Trenaunay–Weber syndrome with temporal lobe astrocytoma Acta Paediatr Scand 68 119 1979.—An 8-year-old boy with a variant of the Klippel–Trenaunay–Weber syndrome (KTW syndrome) is described The hemangiomatous tissue located to the right half of his trunk and extremities was hypotrophic On the same side on his face and gingivae the tissue appeared hypertrophic and dental abnormalities were present Moreover the patient suffered from psychomotor epilepsy caused by a right-sided temporal astrocytoma The connection between the KTW syndrome and the neurocutaneous syndromes is discussed

KEY WORDS Klippel–Trenaunay–Weber psychomotor epilepsy temporal lobe astrocytoma neurocutaneous syndromes

In 1900 Klippel & Trenaunay described the naevus variqueux osteo hypertrophique syndrome This description included as main items cutaneous and subcutaneous haemangiomas phlebectasias and hypertrophy especially of the skeletal bones in the affected areas of the body (5) Some years later Weber reported on a similar entity (10) The close connection between the Klippel–Trenaunay–Weber syndrome (KTW syndrome) and the neurocutaneous syndromes was established early (9)

Subsequently more abnormalities have been added to the KTW syndrome i e macrosyndactyli (6) dental abnormalities (7) and atrophy of the involved areas (4)

The patient described in the following report presents a variant of the KTW syndrome Apart from dystrophy of the haemangiomatous tissue he suffered from a psychomotor epilepsy caused by a temporal astrocytoma located to the affected side

CASE REPORT

The patient an 8-year-old boy had been afflicted since birth by cutaneous telangiectasia resembling a port wine stain of the right half of his body with the exception of his face The angiomatous area was hypoplastic whereas he showed hypertrophy of the right side of his face (Fig 1) The facial asymmetry was not caused by osseus hypertrophy as ascertained by X ray of the skull Dental abnormalities and right sided gingival hyperplasia were present (Fig 2)

No sign of neurocutaneous disease was apparant At 5 months of age focal epileptic seizures occurred Electroencephalography (EEG) showed a right sided focal pathology but both the neurological examination and technetium brain scanning were normal During short term treatment with diphenylhydantoin the seizures disappeared and the EEG became normal At 5 years of age psychomotor epilepsy developed and treatment with carbamazepine was initiated

At the age of eight his epilepsy was aggravating and for the first time he complained of headache Ophthalmological examination was normal X ray of the skull showed distension of the sutures but no calcifications Computer scanning demonstrated a tumour of the right temporal lobe and a carotid arteriography revealed the tumour to be non vascular At craniotomy a 5×4×3 cm well-delimited tumour was found in the medial lower part of the right temporal lobe which was removed in toto Histopathological examination showed an astrocytoma isomor

27 Wildenhoff K E A micromethod for the enzymatic determination of acetate and D-hydroxybutyrate in blood and urine *Scand J Clin Lab Invest* 25 171 1971
28 Williams P R Fiser J H Sperling M A & Oh W Effect of oral alanine feeding on blood glucose plasma glucagon and insulin concentrations in small for gestational age infants *N Engl J Med* 292 612 1975
29 Wise J K Hendler R & Felig P Influence of glucocorticoids on glucagon secretion and plasma amino acids concentrations in man *J Clin Invest* 52 2774 1973

Submitted April 10 1978
Accepted May 23 1978

(L L) Department of Neonatology
Hopital Debrousse
69005 Lyon
France

ostéo-hypertrophique *Arch Gen Med (Paris)* *3* 641 1900
6 Lamar L M Farber G A & O Quinn S E Klippel-Trenaunay-Weber syndrome *Arch Dermatol* *91* 58 1965
7 Miescher G Uber plane Angiome (Naevi hyperaemici) *Dermatologica* *106* 176 1953
8 Peterman A F Hayles A B Dockerty M B & Love J G Encephalotrigeminal angiomatosis (Sturge-Weber disease) *JAMA* *167* 2169 1958
9 Teller H & Lindner B Uber Mischformen der phakomatosen Syndrome von Sturge-Weber und Klippel-Trenaunay *Z Hautkr* *13* 113 1957
10 Weber F P Angioma formation in connection with hypertrophy of limbs and hemihypertrophy *Br J Dermatol* *19* 231 1907

Submitted March 10 1978
Accepted June 10 1978

(P H) Department of Paediatrics G
University of Copenhagen
Rigshospitalet
Blegdamsvej
DK 2100 Ø Copenhagen
Denmark

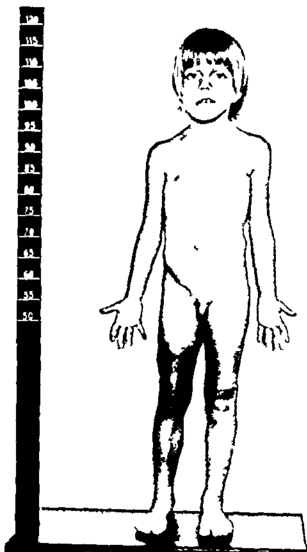


Fig 1 Right sided hypotrophic port wine naevus in an 8 year old boy

phicum piloides Kernohan type I After the operation the psychomotor epilepsy disappeared entirely

DISCUSSION

A KTW syndrome including dystrophy of the haemangiomatous areas of the body and cases with swelling of one half of the face and dental abnormalities have previously been reported (1 4, 7) In addition to the above mentioned symptoms our patient suffered from a psycho motor epilepsy caused by an astrocytoma in the temporal lobe on the same side as the an giomatous tissue

An atypical manifestation of the Sturge-Weber syndrome might be suggested in spite of the lack of facial port wine naevus (8) How



Fig 2 Dental abnormalities and right sided gingival hyperplasia

ever our patient had no intracerebral calcifica tions no ocular involvement and no angioma of the leptomeninges

Because of the known connection between the KTW syndrome and the neurocutaneous syndromes (phacomatoses) (9) a tumour of the cerebrum might be expected in our patient The neuropathological pattern of glial prolifer ation in the phacomatoses results in the possi bility of tumours at every level of the nervous system (2 3) We therefore suggest that the combination of the KTW syndrome and a cerebral astrocytoma as found in our patient is an expected variant of the KTW syndrome To our knowledge our patient represents a combination of syndromes not previously re ported

REFERENCES

1 Belovic B Nethercott J & Donsky H J An unusual variant of Klippel-Trenaunay-Weber syn drome *Can Med Assoc J 111* 439 1974
2 Canale D Bebin J & Knighton R S Neurologic manifestations of von Recklinghausen s disease of the nervous system *Confin Neurol (Basel) 24* 359 1964
3 Chalhub E G Neurocutaneus syndromes in child hood *In* A L Prensky (ed) *Pediatric Neurology* The Pediatric Clinics of North America W B Saun ders Company Philadelphia 1976 p 509
4 Ippen H Quadrantendystrophie mit Gefässanoma lien *Dtsch Med Wochenschr 98* 687 1973
5 Klippel M & Trenaunay P Du nævus variqueux

ostéo hypertrophique *Arch Gen Med (Paris) 3* 641 1900
6 Lamar L M Farber G A & O Quinn S E Klippel–Trenaunay–Weber syndrome *Arch Dermatol 91* 58 1965
7 Miescher G Uber plane Angiome (Naevi hyperaemici) *Dermatologica 106* 176 1953
8 Peterman A F Hayles A B Dockerty M B & Love J G Encephalotrigeminal angiomatosis (Sturge–Weber disease) *JAMA 167* 2169 1958
9 Teller H & Lindner B Uber Mischformen der phakomatosen Syndrome von Sturge–Weber und Klippel–Trenaunay *Z Hautk 13* 113 1952
10 Weber F P Angioma formation in connection with hypertrophy of limbs and hemihypertrophy *Br J Dermatol 19* 231 1907

Submitted March 10 1978
Accepted June 10 1978

(P H) Department of Paediatrics G
University of Copenhagen
Rigshospitalet
Blegdamsvej
DK 2100 Ø Copenhagen
Denmark

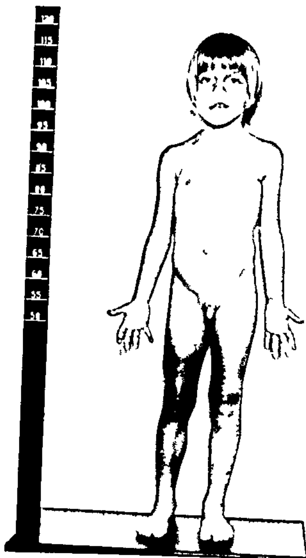


Fig 1 Right sided hypotrophic port wine naevus in an 8 year old boy



Fig 2 Dental abnormalities and right sided gingival hyperplasia

phicum piloides Kernohan type I After the operation the psychomotor epilepsy disappeared entirely

DISCUSSION

A KTW syndrome including dystrophy of the haemangiomatous areas of the body and cases with swelling of one half of the face and dental abnormalities have previously been reported (1 4 7) In addition to the above mentioned symptoms our patient suffered from a psychomotor epilepsy caused by an astrocytoma in the temporal lobe on the same side as the angiomatous tissue

An atypical manifestation of the Sturge–Weber syndrome might be suggested in spite of the lack of facial port wine naevus (8) However our patient had no intracerebral calcifications no ocular involvement and no angioma of the leptomeninges

Because of the known connection between the KTW syndrome and the neurocutaneous syndromes (phacomatoses) (9) a tumour of the cerebrum might be expected in our patient The neuropathological pattern of glial proliferation in the phacomatoses results in the possibility of tumours at every level of the nervous system (2 3) We therefore suggest that the combination of the KTW syndrome and a cerebral astrocytoma as found in our patient is an expected variant of the KTW syndrome To our knowledge our patient represents a combination of syndromes not previously reported

REFERENCES

1 Belovic B Nethercott J & Donsky H J An unusual variant of Klippel–Trenaunay–Weber syndrome *Can Med Assoc J 111* 439 1974
2 Canale D Bebin J & Knighton R S Neurologic manifestations of von Recklinghausen s disease of the nervous system *Confin Neurol (Basel) 24* 359 1964
3 Chalhub E G Neurocutaneus syndromes in childhood *In* A L Prensky (ed) *Pediatric Neurology* The Pediatric Clinics of North America W B Saunders Company Philadelphia 1976 p 509
4 Ippen H Quadrantendystrophie mit Gefassanomalien *Dtsch Med Wochenschr 98* 68? 1973
5 Klippel M & Trenaunay P Du nævus variqueux

Acta Pædiatr Scand 68 123–128 1979

CASE REPORT

A VARIANT FORM OF 2 METHYL 3 HYDROXYBUTYRIC AND 2 METHYLACETOACETIC ACIDURIA

S HALVORSEN [1] O STOKKE and E JELLUM

From the Institute of Paediatric Research and the Institute of Clinical Biochemistry Rikshospitalet University of Oslo Oslo Norway

ABSTRACT Halvorsen S Stokke O and Jellum E (Institute of Paediatric Research and Institute of Clinical Biochemistry Rikshospitalet University of Oslo Oslo Norway) A variant form of 2-methyl 3-hydroxybutyric and 2-methylacetoacetic aciduria Acta Pædiatr Scand 68 123 1979 —A new case of assumed β ketothiolase deficiency excreting 2 methyl 3 hydroxybutyrate and tiglylglycine is described in a 15 year-old boy The patient presented with episodes of metabolic acidosis following intercurrent infections in the early childhood After the age of 7 years he has had periods of headache but no acidotic episodes have occurred even during infections Systematic dietary treatment has not been instituted and the patient is physically and mentally normal This indicates a mild variant of the β ketothiolase deficiency Diagnosis of the condition may be obscured by large quantities of ordinary ketone bodies and requires gas chromatographic and mass spectrometric techniques

KEY WORDS β ketothiolase deficiency 2-methyl 3 hydroxybutyrate tiglylglycine metabolic acidosis ketoacidosis gas chromatography mass spectrometry

The clinical pictures of the 6 previously published patients with 2 methyl 3 hydroxybutyric and 2 methylacetoacetic aciduria (β ketothiolase deficiency) have varied widely In the patient described by Hillman & Keating (3) vomiting and acidosis started a few days after birth In the four patients described by Daum et al (1) the symptoms started in the second year of life with episodes of vomiting and metabolic acidosis while in the patient described by Gompertz et al (2) the first and only episode of acidosis occurred at the age of 7 years Between the ages of 2 and 7 years this patient had presented with frequent episodes of head ache abdominal pain and vomiting but none of these episodes had given any cause for concern

The patient reported in this paper had episodes of metabolic acidosis between 1 and 4 years of age but later in childhood he developed episodes of headache The purpose of the present report is to describe the clinical picture of this variant of the thiolase deficiency and to point out that the heterogeneity in this disease probably is as marked as in maple syrup urine disease (MSUD) Further it is the purpose to describe the pattern of metabolites and some difficulties related to the diagnosis of the disorder The metabolic steps leading to the formation of the abnormal compounds are shown in Fig 1

CASE REPORT

T K a boy born February 9 1963 was admitted 4 times to the Department of Pediatrics Rikshospitalet Oslo for coma and/or acidosis There is no consanguinity between the parents An older brother is healthy

His mother grandmother and other female members of the mother s family have hemicrania None have had periodic vomiting in childhood or other known metabolic diseases

Pregnancy delivery and neonatal period were uneventful The psychomotor development was within normal limits

[1] Present address Paediatric Department Ullevål Hospital Oslo Norway

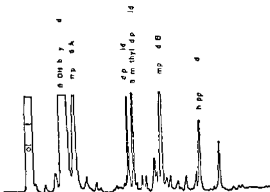
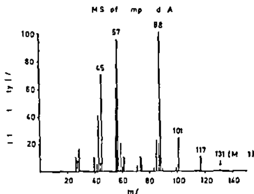


Fig 2 Gas chromatographi separation of organic acids in the urine of the patient The urine was acidified extracted with diethyl ether and the organic acids were converted into methyl esters with diazomethane prior to separation on a 6 ft ×1/4 in glass column filled with 10% OV 17 on Gas Chrom Q The temperature was programmed from 80 to 280 at a rate of 6 per minute



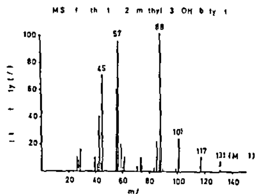


Fig 3 Mass spectra of compound A and of authentic 2 methyl 3 hydroxybutyric acid (methyl esters)

ing a urine sample collected during a period of severe metabolic a idosis (second admission) The presence in the urine of large quantities of β hydroxybutyric acid (Fig 2) was evident Elevated amounts of adipic acid was also noted (Fig 2) as is usual in ketoacidotic patients (6) In addition to the above metabolites the propositus presented with abnormal compounds (denoted A and B in Fig 2) in his urine The mass spectrum of compound A (Fig 3 top) suggested the structure to be 2 methyl 3 hydroxybutyric acid (methyl ester) In order to verify this structure the authentic compound was synthesized from acetaldehyde and 2 bromopropionic acid using a classical Reformatsky reaction The synthetic 2 methyl 3 hydroxy butyric acid methyl ester co chromatographed with compound A from the patient using two different gas chromatography columns (10% OV 17 and 8% BDS) Furthermore the mass spectrum of authentic 2 methyl 3 hydroxybutyric acid methyl ester and of compound A were identical (Fig 3) Thus it was established that the patient excreted considerable quantities of methyl 3 hydroxy butyric acid in his urine

We also looked carefully for the corresponding keto acid (methylacetoacetic acid) using gas chromatography and mass spectrometry without finding this metabolite Even when the methoxime–methyl esters were prepared directly from the patient s urine we failed to find the methylacetoacetic acid

The mass spectrum of compound B suggested its structure to be either tiglylglycine or 3 methylcrotonylglycine These compounds have rather similar mass spectra as shown in Fig 4 However the relative intensities of some of the fragments differ and it is clearly seen that the unknown compound B and authentic tiglylglycine had identical mass spectra both being slightly different from that of 3 methylcrotonylglycine methyl ester Final verification of the identity of compound B was done by isothermal gas chromatography using a polar column (BDS) which in contrast to the less polar columns (OV 17 SE 30) readily separates tiglylglycine and 3 methylcrotonylglycine methyl esters As shown in Fig 5 the unknown compound B and tiglylglycine methyl ester co-chromatographed completely eluting well ahead of the methyl ester of 3 methylcrotonylglycine Again the mass spectra of compound B and tiglylglycine (methyl ester) were identical thus establishing the structure of the second abnormal metabolite in the urine of the patient

Quantitative analyses of tiglylglycine and 2 methyl 3 hydroxybutyric acid were carried out by means of gas chromatography Glutaric acid which was not present in native urine was added as internal standard Repeated extractions of the acidified urine samples with methyl acetate were carried out before conversion into the methyl esters and separation in the GC Table 1 shows that approximately equal quantities of tiglylglycine and 2 methyl 3 hydroxybutyric acid were excreted in the urine of the patient The amounts present in the urines varied from about 0 10 mg per g of creatinine in good clinical phases up to 3 mg per g of creatinine during crisis with metabolic acidosis Neither the parents nor a healthy brother excreted detectable amounts of the two metabolites which are not present normally

The concentrations of amino acids in serum and urine have been within normal limits except for an increased

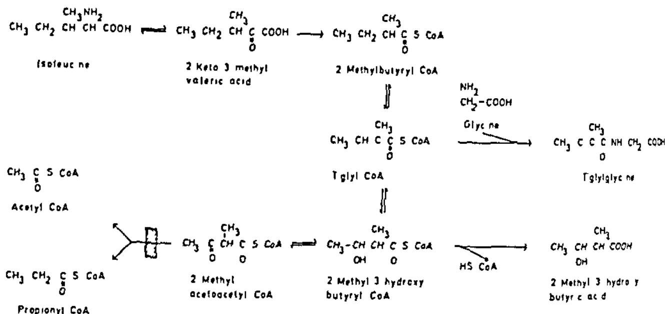


Fig 1 Metabolic fate of isoleucine A block is indicated at the level of β ketothiolase resulting in accumulation of tiglylglycine and 2 methyl 3 hydroxybutyric acid

First admission Fourteen months old he had as others in the neighbourhood diarrhea and vomiting diagnosed as gastroenteritis During the first 2 days of his illness he was conscious but on the third day he went into sopor which gradually developed to coma On admission to Rikshospitalet he was in deep coma and did not react to painful stimuli The respiration was deep frequent and with a sweet odour from the expired air He was pale dehydrated and hypotonic The tendon reflexes were weak He was restless moved his arms and hands constantly and gave the impression of encephalitis

The laboratory investigations revealed a marked metabolic acidosis with an arterial blood pH of 6 82 and both standard bicarbonate and P_{CO} were very low The serum electrolytes were normal on admission He was treated with lactate for 24 hours without success He was then given THAM and bicarbonate and the acidosis improved and was normalized 72 hours after admission During the following days the clinical condition slowly improved but it was apparent that the encephalopathy continued for a long period following normalization of the acidosis EEG was markedly pathological with generalized dysrythmia

The urine showed proteinuria and ketonuria The 2 4 dinitrophenylhydrazine reaction was positive

During the following year the mother observed deep respiration during periods of upper respiratory infections and fever but on these occasions the patient improved without further treatment

Second admission At 3 1/3 years of age he was readmitted for measles and because he had deep and frequent respiration and sopor Blood pH was 7 05 standard bicarbonate 8 mmol/l and P_{CO} 17 mmHg This time he was treated with THAM and bicarbonate from the beginning and within 24 hours the acidosis was normalized The sweet odour from the respiratory air was not noted this time

Third admission 3 1/2 years old he had an upper respiratory infection and slight changes in respiration On admission there were no objective signs of acidosis and the pH and standard bicarbonate were only slightly reduced

Fourth admission At 4 years of age he was admitted the last time for a suspected acidotic episode when he had mumps and a generalized lymph node enlargement which was not well explained He was conscious and had no respiratory changes pH was 7 44 standard bicarbonate 18 mmol/l and P_{CO} 21 mmHg

During the following years he had no episodes of acidosis even when suffering from upper respiratory infections with temperatures in the range of 39°C From the age of 7 years he had episodes with headache The headache usually came in the morning and lasted 3–4 hours It was localized in the front in the midline and usually terminated with vomiting Neurological and ophthalmological examination did not reveal any pathological findings The patient is now 15 years old and both mentally and physically normal

Identification of abnormal compounds in the urine of the patient

Urine samples collected during the clinical episodes were examined using gas chromatography (GC) and mass spectrometry (MS) (4) The acidified urine was extracted with diethyl ether and the extract was methylated with diazomethane An aliquot of the organic acid methyl esters was injected into a Varian CH7 combined GC MS (Varian MAT Bremen Germany) The GC column (6 ft × 1/8 in) was filled with 10% OV 17 on Gas Chrom Q or with 8% BDS on Chromosorb W The GC MS was connected on line to a computer system (Varian Spectrosystem 100 MS)

Fig 2 shows the gas chromatogram obtained by analys

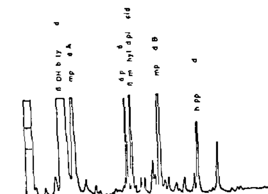
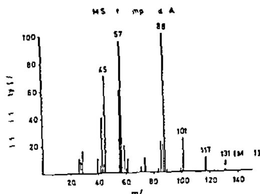


Fig 2 Gas chromatographic separation of organic acids in the urine of the patient The urine was acidified extracted with diethyl ether and the organic acids were converted into methyl esters with diazomethane prior to separation on a 6 ft × 1/4 in glass column filled with 10% OV 17 on Gas Chrom Q The temperature was programmed from 80° to 80° at a rate of 6 per minute



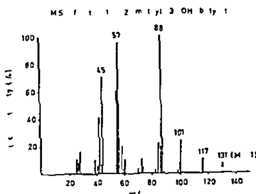


Fig 3 Mass spectra of compound A and of authentic ? methyl 3 hydroxybutyric acid (methyl esters)

ing a urine sample collected during a period of severe metabolic acidosis (second admission) The presence in the urine of large quantities of β hydroxybutyric acid (Fig ?) was evident Elevated amounts of adipic acid was also noted (Fig 2) as is usual in ketoacidotic patients (6) In addition to the above metabolites the propositus presented with 2 abnormal compounds (denoted A and B in Fig 2) in his urine The mass spectrum of compound A (Fig 3 top) suggested the structure to be 2 methyl 3 hydroxybutyric acid (methyl ester) In order to verify this structure the authentic compound was synthesized from acetaldehyde and ? bromopropionic acid using a classical Reformatsky reaction The synthetic 2 methyl 3 hydroxy butyric acid methyl ester co chromatographed with compound A from the patient using two different gas chromatography columns (10% OV 17 and 8% BDS) Further more the mass spectrum of authentic 2 methyl 3 hydroxybutyric acid methyl ester and of compound A were identical (Fig 3) Thus it was established that the patient excreted *considerable quantities of ? methyl 3 hydroxy* butyric acid in h s urine

We also looked carefully for the corresponding keto acid (methylacetoacetic acid) using gas chromatography and mass spectrometry without finding this metabolite Even when the methoxime–methyl esters were prepared directly from the patient s urine we failed to find the methylacetoacetic acid

The mass spectrum of compound B suggested its structure to be either tiglylglycine or 3 methylcrotonylglycine These compounds have rather similar mass spectra as shown in Fig 4 However the relative intensities of some of the fragments d ffer and it is clearly seen that the unknown compound B and authentic tiglylglycine had identical mass spectra both being slightly d fferent from that of 3 methylcrotonylglycine methyl ester Final verification of the identity of compound B was done by isothermal gas chromatography using a polar column (BDS) which in contrast to the less polar columns (OV 17 SE 30) readily separates tiglylglycine and 3 methylcrotonylglycine methyl esters As shown in Fig 5 the unknown compound B and tiglylglycine methyl ester co-chromatographed completely eluting well ahead of the methyl ester of 3 methylcrotonylglycine Again the mass spectra of compound B and tiglylglycine (methyl ester) were identical thus establishing the structure of the second ab*normal metabolite in the urine of the patient*

Quantitative analyses of tiglylglycine and methyl 3 hydroxybutyric acid were carried out by means of gas chromatography Glutaric acid which was not present in native urine was added as internal standard Repeated extractions of the acidified urine samples with methyl acetate were carried out before conversion into the methyl esters and separation in the GC Table 1 shows that approximately equal quantities of tiglylglycine and ? methyl 3 hydroxybutyric acid were excreted in the urine of the patient The amounts present in the urines varied from about 0 30 mg per g of creatinine in good clinical phases up to 3 mg per g of creatinine during crisis with metabolic acidosis Neither the parents nor a healthy brother excreted detectable amounts of the two metabolites which are not present normally

The concentrations of amino acids in serum and urine have been within normal limits except for an increased

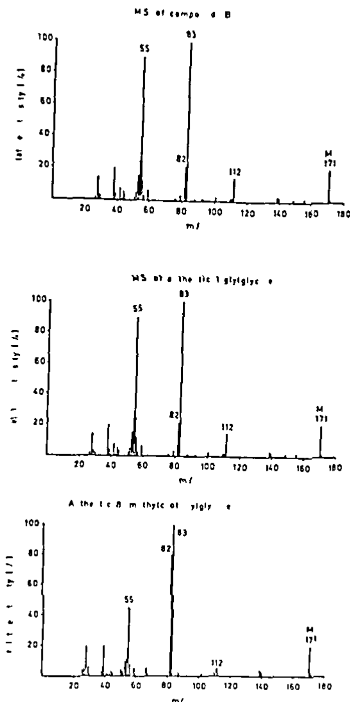


Fig 4 Mass spectra of compound B of authentic tiglyl glycine and of 3 methylcrotonylglycine

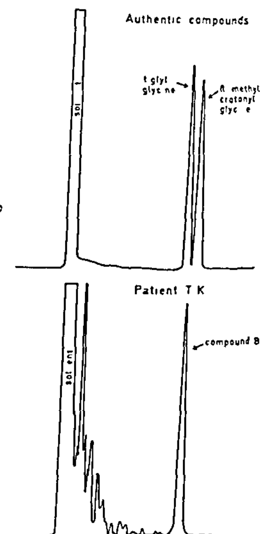


Fig 5 Isothermal gas chromatographic separation of ti glylglycine and β methylcrotonylglycine (top) and of the urinary organic acids in the patient (bottom) The methy esters were prepared as in Fig 2 The GC column (6 ft × 1/4 in) was filled with 8% BDS on Chromosorb W and the column temperature was kept constant at 170

glycine level 4 3 μmol/mg creatinine in the urine sample from the first admission

DISCUSSION

The clinical picture of our patient is charac terized by a few episodes of severe metabolic acidosis in infancy with symptoms and signs mimicking those of encephalitis Apart from these episodes which were precipitated by gastroenteritis and upper respiratory tract in fections the patient has developed normally From 7 years of age he has occasionally had periods with headache

Various 5 carbon fatty acids e g isovaleric and tiglic acids and several of the correspond ing hydroxy acids have been found to exert encephalopathic effects in rabbits (7) Al though no data exist for the toxicology of the metabolites found in our patient it is reason able to believe that they are at least in part responsible for the metabolic encephalopathy present

Table 1 *Concentration of abnormal metabolites in the urine related to the excretion of creatinine*

At 11 5/12 years of age the creatinine excretion was 665 mg/24 h

Age (years)		methyl 3 hydroxy butyric acid (mg/g creat)	Tiglyl glycine (mg/g creat)
1 /12	After treatment	0 51	0 8
3 4/1	Day of admission	2 94	1 01
	2nd day	1 78	1 39
	2nd day	1 34	2 40
	3rd day	0 74	0 67
	4th day	0 48	0
11 5/12		0 15	0 18
11 7/12		0 20	0 10

ache might possibly be a late complication of this metabolic encephalopathy It could also be caused by an acute increase in the level of the abnormal metabolites However no increased excretion of the metabolites could be found in the urine in relation to the headache making this hypothesis less likely

The 7 cases with this disease published so far show a wide variation of severity from life threatening acidosis shortly after birth to one single episode of acidosis as late as at 7 years of age The situation is thus similar to that of MSUD where there are several variants and where each case or family almost has its own clinical picture This indicates that 2 methyl 3 hydroxybutyric aciduria must be suspected in every case of chronic or intermittent acidosis in infancy or childhood In the individual patient the episodes may also vary markedly and as in MSUD it appears as if the most severe episodes occur following gastroenteritis

Although these patients have a sweet odour it is much less characteristic than the curry like odour of MSUD or that of isovaleric acidemia By the standard clinical chemical urine analyses it is impossible to distinguish between an ordinary ketoacidosis with the excretion of 3 hydroxybutyrate acetoacetate and acetone and the present disorder with the excretion of the corresponding 2 methyl acids At present a gas chromatographic analysis of either urine or serum is needed in order to make the diagnosis

Differences in the redox situation in the cells may give rise to different ratios between hydroxy and keto acids Our patient had apparently displaced the redox state in the direction of the reduced form explaining the absence of both 2 methylacetoacetate and acetoacetate

Even by gas chromatography the diagnosis of a β ketothiolase deficiency may be difficult The chromatographic properties of 3 hydroxybutyrate and 2 methyl 3 hydroxybutyrate are closely related making the compounds difficult to separate from each other Patients with ordinary ketoacidosis excrete small amounts of 2 methyl 3 hydroxybutyrate in addition to the well known ketone bodies (5) During the acute episodes our patient with a probable β ketothiolase deficiency excreted larger amounts of 3 hydroxybutyrate than of the typical metabolite 2 methyl 3 hydroxybutyrate This points to some sort of mutual inhibition of the corresponding enzymic steps in the two pathways However the finding of large amounts of 2 methyl 3 hydroxybutyrate together with tiglylglycine is a characteristic feature In addition these metabolites are present between the acute episodes also when the ordinary ketone bodies are lacking

REFERENCES

1 Daum R S Scriver C R Mamer O A Delvin E Lamm P & Goldman H An inherited disorder of isoleucine catabolism causing accumulation of α methylacetoacetate and α methyl β-hydroxybutyrate and intermittent metabolic acidosis *Pediatr Res* 7 149 1973

Gompertz D Saudubray J M Charpentier C Bartlett K Goodey P A & Draffan G H A defect in L isoleucine metabolism associated with α methyl β-hydoxybutyric and α methylacetoacetic aciduria Quantitative in vivo and in vitro studies *Clin Chim Acta* 57 69 1974

3 Hillman R H & Keating J P Beta ketothiolase deficiency as a cause of the "ketotic hyperglycinemia syndrome *Pediatrics* 53 221 1974

4 Jellum E Stokke O & Eldjarn L Combined use of gas chromatography mass spectrometry and computer in diagnosis and studies of metabolic disorders *Clin Chem 18* 800 1972
5 Landaas S Accumulation of 3 hydroxyisobutyric acid 2 methyl 3 hydroxybutyric acid and 3 hydroxy isovaleric acid in ketoacidosis *Clin Chim Acta 64* 143 1975
6 Pettersen J E Jellum E & Eldjarn L The occurrence of adipic and suberic acid in urine from ketotic patients *Clin Chim Acta 38* 17 1972
7 Teychenne P F Walters L Claveria L E Calne D B Price J MacGillivary B B & Gompertz D The encephalopathic action of five-carbon atom fatty acids in the rabbit *Clin Sci Mol Med 50* 463 1976

Submitted March 31 1978
Accepted May 22 1978

(S H) Department of Paediatrics
Ullevål Sykehus
Oslo 1
Norway

Acta Pædiatr Scand 68 129–132 1979

CASE REPORT

THE ROLE OF ZINC IN TOTAL PARENTERAL NUTRITION

N PRINCIPI A GIUNTA and A GERVASONI

From the Department of Paediatrics (II) of Milano University Medical School Milano Italy

ABSTRACT Principi N Giunta A and Gervasoni A (Department of Paediatrics (II) of Milano University Medical School Milano Italy) The role of zinc in total parenteral nutrition Acta Paediatr Scand 68 129 1979 —Zinc deficiency was observed in an infant receiving total parenteral nutrition (TPN) for chronic untractable diarrhoea Clinical findings included low zinc plasma levels skin lesions and loss of all the advantages of TPN such as weight gain serum proteins and albumin increase and normalization of intestinal mucosa Oral administration of zinc sulphate was the decisive factor making possible both the improvement of clinical and laboratory findings and alimentation by natural route

KEY WORDS Zinc deficiency total parenteral nutrition

Total parenteral nutrition (TPN) may ensure excellent results only if the infusion contains all the needed nutrients in appropriate amounts (5) A case we recently observed demonstrates the critical role of an adequate zinc supply

MATERIALS AND METHODS

The patient

R S was born to healthy unrelated parents after a full term uneventful pregnancy He weighed 3 4 kg Neonatal period was normal and he was fed cow s milk formula Diarrhoea 6 to 15 times per day began at one month of age Dietary and antibiotic therapy was unsuccessful At ? months and 15 days of age he was admitted to our department Physical examination revealed a poorly nourished infant weighing 3 ? kg and measuring 56 cm the circumference of his head was 36 cm Repeated stool cultures revealed no pathogens During the first 40 days a lactose free diet with soya milk peripheral intravenous infusions of glucose and aminoacids and antibiotics were given without effect on his diarrhoea At the end of this period the body weight was 3 3 kg

TPN was then started About 150 ml/kg of a prepared fat free solution were administered daily through a silastic catheter placed in the superior vena cava Total calorie intake was about 1 0 calories/kg/die protein administration 3 g/kg/die and zinc supply 40 μg/kg/die Essential fatty acids were ensured with daily application of sun flower seed oil to the skin Plasma or when necessary fresh blood was transfused once weekly at the dosage of ?0 ml/kg A sharp and constant increase of body weight along with the cessation of the diarrhoea was observed At the end of the first month of TPN the weight increase stopped and skin lesions appeared They started as a moist eczematoid area in the nasolabial folds and progressed to crusting followed by bullous or pustular lesions all over the face (Fig 1*a*) and around the other natural orifices eventually coalescing to form large erosive areas Some days later a significant alopecia was also noticed In this period an attempt of oral feeding with a lactose free cow s milk formula produced voluminous diarrhoea and a loss of weight in spite of the intravenous administration of the same volume of nutritive solution On the 51st day of TPN oral administration of zinc sulphate 25 mg/day was started Within 5 days a dramatic improvement of skin lesions (Fig 1*b*) and a resumption of weight gain occurred On the 56th day of TPN the dislodgement of the catheter from the cutdown site led to the discontinuation of TPN A lactose free cow s milk formula was then given while zinc administration at the same dosage continued Oral feeding was well tolerated no diarrhoea appeared even though 14 days later zinc supply was discontinued Skin lesions never recurred and the patient s general condition gradually improved

Laboratory evaluation

Samples of blood were collected before the beginning of TPN and several times during and after its cessation In all samples serum total proteins and albumin zinc and alkaline phosphatase activity were determined Oral jejunal mucosal biopsies were performed at the beginning and on the ?7th day of TPN when the skin lesions appeared and when a good tolerance of oral feeding was obtained A Crosby Kugler capsule was used and the specimens stained with hematoxylin and eosin

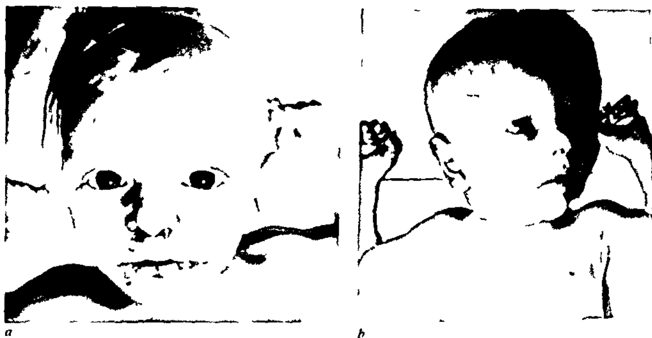


Fig 1 (*a*) Skin lesions after one month of TPN when zinc plasma concentration was 27 µg/dl (*b*) Regression of skin lesions after zinc sulphate oral administration

RESULTS

Before the beginning of TPN serum total proteins and albumin were very low while alkaline phosphatase activity was normal Zinc plasma level was a little below the normal range (Fig 2) The small bowel biopsy revealed a f mucosa with hyperplastic crypts In the fi month of TPN the normalization of serum p tein concentration and a significant impro ment of the intestinal picture (Fig 3*a*) w observed When zinc plasma levels beca

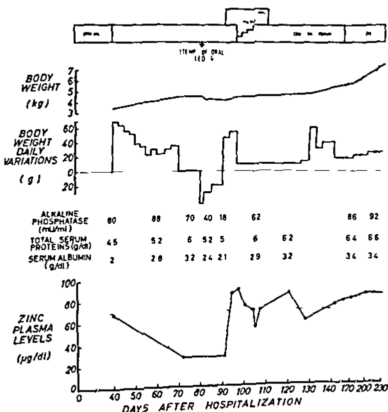


Fig 2 Clinical course and biochem parameters of the studied patient

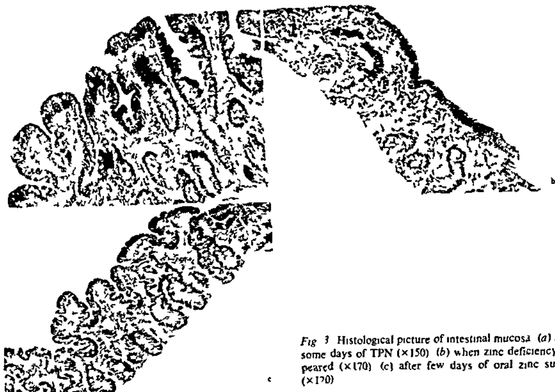


Fig 3 Histological picture of intestinal mucosa (a) after some days of TPN (×150) (b) when zinc deficiency appeared (×170) (c) after few days of oral zinc supply (×170)

markedly depressed a reduction both of serum protein concentration and of alkaline phosphatase activity was noticed (Fig 2) In addition intestinal mucosal lesions returned similar to those seen when malnutrition was present (Fig 3b) With oral administration of zinc sulphate all the studied biochemical parameters returned to normal values The last small bowel biopsy demonstrated only a slightly abnormal mucosa (Fig 3c)

DISCUSSION

This case clearly underlines the critical importance of an adequate zinc supply in conditioning TPN efficacy Clinical and laboratory findings during TPN were as expected for as long as zinc plasma concentration remained near the normal range When it decreased to a very low level and was accompanied by skin lesions an interruption of weight gain a drop in serum protein concentration and a reappearance of a flat intestinal mucosa were manifest Zinc sulphate administration was the decisive factor making possible both the improvement of clinical and laboratory findings and the alimentation by natural route

The importance of zinc in nucleic acid metabolism and protein synthesis is well demonstrated in many experimental studies (2 10 12) in infants and children an inadequate zinc intake may retard growth sexual development and wound healing (9 13) and may induce anorexia impaired taste perception pica and lethargy (3 4) However the best clinical example of zinc deficiency is acrodermatitis enteropathica (8) a disease in which both skin lesions and bowel alterations may be related to a reduced protein synthesis and cellular turnover due to a lack of this element (7) It is reasonable to suggest that the same metabolic impairment may have taken place in this patient leading despite an adequate aminoacid intake to a reduction of serum protein concentration and a regression of intestinal mucosa to atrophy

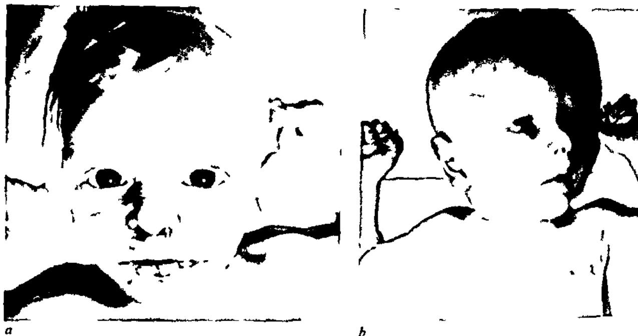


Fig 1 (*a*) Skin lesions after one month of TPN when zinc plasma concentration was 27 µg/dl (*b*) Regression of skin lesions after zinc sulphate oral administration

RESULTS

Before the beginning of TPN serum total proteins and albumin were very low while alkaline phosphatase activity was normal Zinc plasma level was a little below the normal range (Fig 2) The small bowel biopsy revealed a mucosa with hyperplastic crypts In the f month of TPN the normalization of serum p tein concentration and a significant impro ment of the intestinal picture (Fig 3*a*) w observed When zinc plasma levels beca

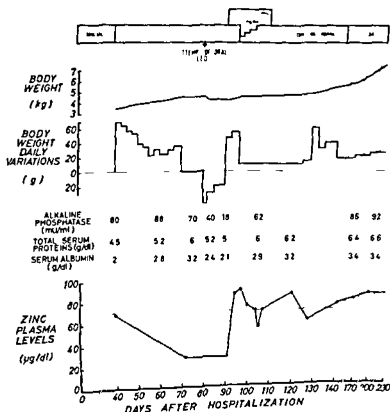


Fig 2 Clinical course and biochem par... of the studied patient

Acta Pædiatr Scand 68 133–135 1979

CASE REPORT

IMMUNO DEFICIENCY IN SCHWARTZ JAMPEL SYNDROME

F MOLLICA [1] A MESSINA [2] F STIVALA[2] and L PAVONE[1]

From the Department of Paediatrics and the [2]Department of General Pathology University of Catania Italy

ABSTRACT Mollica F Messina A Stivala F and Pavone L (Departments of Paediatrics and of General Pathology University of Catania Italy) Immunodeficiency in Schwartz Jampel syndrome Acta Paediatr Scand 68 133 1979 —Two sisters born in a consanguineous marriage and affected by Schwartz Jampel syndrome had a complex im munodeficiency involving not only the humoral but also the cellular immune response

KEY WORDS Schwartz Jampel syndrome immunodeficiency

ecently (6) a selective IgA deficiency was ound in the serum and external secretions of girl affected by Schwartz Jampel syndrome S J s) a rare disease characterized by typical acies muscle and skeletal abnormalities and rowth retardation Immunological studies erformed in two of our patients with this syn lrome did not reveal Ig deficiencies but a nore complex immunological deficiency

CASE REPORTS

The two patients were sisters born in a consanguineous marriage

L P has shown progressive modification of her facial appearance and contractures of several muscular groups from the second year of life and progressive muscle weakness and walking difficulty from the third year No abnormal susceptibility to infections is reported by the parents On admission the girl is 7 years old Relevant clinical data are a stature of 110 cm (below the third centile) micrognathia narrowed palpebral fissures small mouth long philtrum (Fig 1) severe myopia high pitched vo ce pidgeon breast deformity contractures of several muscul r groups joint limitation kyphoscoliosis and stiff ga t A typical myotonic response is found in the electromyogram

Results of routine laboratory tests are normal except for a low serum albumin/globulin ratio (0 66) and a moderate increase of gammaglobulins (4) There is lack of re ponse of growth hormone secretion evaluated by radioimmunoassay to arginine and insulin stimulation An intradermal test with 10 I U of PPD (from Berna Institute) i strongly positive although the girl has not been vaccinated against tuberculosis and there is no history of tuberculo is

S P has shown modification of the face by the end of the second year and unsteady gait and progressive loss of muscular strength by the age of 2½ years She had not experienced frequent infections At 5½ years she is 103 cm tall (below the 3rd centile) and has a face very similar to



Fig 1 The face of a girl with Schwartz Jampel syndrome

Few data are available on minimum intravenous daily zinc requirement. It is however well known that if during TPN zinc is not added to the nutritive solution a deficiency syndrome may appear (1). The demonstration that in our patient zinc deficiency appeared despite an intake of 40 μg/kg/die suggests that even this amount usually recommended in guidelines for TPN (14) may be insufficient. This conclusion is in complete agreement with the data of Ricour et al. (11) and James & MacMahon (6) who demonstrated that during TPN the decline of zinc plasma level can be prevented only by providing term infants and small premature babies with 100 and 200 μg/kg/die respectively.

It is however likely that intravenous zinc requirement may differ significantly from patient to patient according to individual body stores and the degree of anabolism of the first days of TPN. It is important therefore, when TPN is performed in infants and children with severe malnutrition, to frequently monitor zinc plasma level and eventually increase daily zinc supply if all the advantages of TPN are to be maintained.

REFERENCES

1. Arakawa T, Tamura T, Igarashi Y, Suzuki H & Sandstead H H. Zinc deficiency in two infants during total parenteral alimentation for diarrhea. *Am J Clin Nutr 29*: 197, 1976.
2. Fosmire G J, Al Ubaidi Y Y & Sandstead H H. Some effects of postnatal zinc deficiency on developing rat brain. *Pediatr Res 9*: 89, 1975.
3. Hambidge K M, Hambidge C, Jacobs M & Baum J D. Low levels of zinc in hair, anorexia, poor growth and hypogeusia in children. *Pediatr Res 6*: 868, 1972.
4. Hambidge K M & Silverman A. Pica with rapid improvement after dietary zinc supplementation. *Arch Dis Child 48*: 567, 1973.
5. Heird W C & Winters R W. Total parenteral nutrition: the state of the art. *J Pediatr 86*: 2, 1975.
6. James B E & MacMahon R W. Balance studies of nine elements during complete intravenous feeding of small premature infants. *Aust Paediatr J 1*: 154, 1976.
7. Kelly R, Davidson G P, Townley R R W & Campbell P E. Reversible intestinal mucosal abnormality in acrodermatitis enteropathica. *Arch Dis Child 51*: 219, 1976.
8. Moynahan E J. Acrodermatitis enteropathica: a lethal inherited human zinc deficiency disorder. *Lancet II*: 339, 1974.
9. Pories W H, Henzel J H, Rob C G & Strain W H. Acceleration of healing with zinc sulphate. *Ann Surg 165*: 432, 1967.
10. Prasad A S & Oberleas D. Thymidine kinase activity and incorporation of thymidine into DNA in zinc-deficient tissues. *J Lab Clin Med 83*: 634, 1974.
11. Ricour C, Duhamel J F, Gros J, Maziere B & Comar D. Oligo éléments chez l'enfant en nutrition parentérale exclusive. *Arch Franç Ped 34*: 47, 1977.
12. Sandstead H H, Gillespie D D & Brady R N. Zinc deficiency: effect on brain of the suckling rat. *Pediatr Res 6*: 119, 1972.
13. Sandstead H H, Prasad A S, Schulert A R, Farid Z, Miale A, Bassilly S & Darby W J. Human zinc deficiency: endocrine manifestations and response to treatment. *Am J Clin Nutr 20*: 422, 1967.
14. Shils M E. Guidelines for total parenteral nutrition. *JAMA 220*: 1721, 1972.

Submitted Febr. 28, 1978
Accepted June 25, 1978

(N. P.) Department of Paediatrics
Istituti Clinici di Perfezionamento
Via Commenda 9
20122 Milano
Italy

Acta Pædiatr Scand 68 133–135 1979

CASE REPORT

IMMUNO DEFICIENCY IN SCHWARTZ JAMPEL SYNDROME

F MOLLICA[1] A MESSINA[2] F STIVALA[2] and L. PAVONE[1]

From the Department of Paediatrics and the [2]Department of General Pathology University of Catania Italy

ABSTRACT Mollica F Messina A Stivala F and Pavone L (Departments of Paediatrics and of General Pathology University of Catania Italy) Immunodeficiency in Schwartz Jampel syndrome Acta Paediatr Scand 68 133 1979 —Two sisters born in a consanguineous marriage and affected by Schwartz Jampel syndrome had a complex im munodeficiency involving not only the humoral but also the cellular immune response

KEY WORDS Schwartz Jampel syndrome Immunodeficiency

Recently (6) a selective IgA deficiency was found in the serum and external secretions of a girl affected by Schwartz Jampel syndrome (S J s) a rare disease characterized by typical facies muscle and skeletal abnormalities and growth retardation Immunological studies performed in two of our patients with this syn drome did not reveal Ig deficiencies but a more complex immunological deficiency

CASE REPORTS

The two patients were sisters born in a consanguineous marriage

L P has shown progressive modification of her facial appearance and contractures of several muscular groups from the second year of life and progressive muscle weakness and walking difficulty from the third year No abnormal susceptibility to infections is reported by the parents On admission the girl is 7 years old Relevant clinical data are a stature of 110 cm (below the third cent le) micrognathia narrowed palpebral fissures small mouth long philtrum (Fig 1) severe myopia high pitched voice pidgeon breast deformity contractures of several mu cular groups joint limitation kyphoscoliosis and stiff gait A typical myotonic response is found in the electromyogram

Results of routine laboratory tests are normal except for a low serum albumin/globulin ratio (0 66) and a moder ate increase of gammaglobulins (4) There is lack of response of growth hormone secretion evaluated by radioimmunoassay to arginine and insulin stimulation An intradermal test with 10 I U of PPD (from Berna Institute) is trongly positive although the girl has not been vac cinated against tuberculosis and there is no history of tuberculosis

S P has shown modification of the face by the end of the second year and unsteady gait and progressive loss of muscular strength by the age of 2½ years She had not experienced frequent infections At 5½ years she is 103 cm tall (below the 3rd centile) and has a face very similar to



Fig 1 The face of a girl with Schwartz Jampel syndrome



Fig 2 The sister of the patient in Fig 1

Table 1 *Immunological studies in 2 sisters with the Schwartz Jampel syndrome*

	Case 1 P L	Case 2 P S	Normal range in this laboratory
IgG g/l*	15 01	22 60	8 0 16 8
IgA g/l	1 87	3 32	1 4-4 2
IgM g/l*	3 76	2 88	0 5-1 9
IgE mg/l*	0 35	0 32	0 3-0 6
C 3 g/l	0 65	0 60	0 55 1 34
Leukocytes 10⁹/l	4 1	3 3	5 0-9 0
Lymphocytes 10⁹/l	1 64	1 06	1 5-2 7
% stimulation by PHA^c	61	52	70-80
% E rosette forming cells (T lymphocytes)	16	14 8	55 65
% EA rosette forming cells (B lymphocytes)	5 5	10 0	15-20

Immunodiffusion technique using plates from Behring werke AG (Marburg Lahn)

^b Radioimmunoassay

^c Mononuclear cell cultures were obtained according Böyum (2) A previously established optimal dose of 0 μg/ml of PHA was used for stimulation E and EA roset forming cells were evaluated by the techniques of Jond et al (5) and of Hallberg et al (4) respectively

that of her sister with narrowed eyelids small mouth and sharply pointed chin (Fig 2) There are thoracic deformity nasal voice muscle contractures kyphoscoliosis and a hampered gait Electromyogram shows a typical myotonic response An intradermal PPD test (10 I U) is negative

Laboratory findings are normal with the exception of unresponsiveness of serum growth hormone levels to post arginine and post insulin hypoglycaemia The results of the immunological study of the sisters are reported in Table 1 The same tests performed in both parents give normal results

DISCUSSION

Selective IgA deficiency is the most frequent immunodeficiency It has been found in 0 7% of an unselected population (3) It may be found in healthy subjects and in association with various and apparently unrelated diseases Dr Kirschner & Pachman (6) reported a 3½ year old girl with S J s who showed frequent infections and a selective IgA deficiency Another subject with this syndrome (1) showed frequent respiratory tract infection but in this patient no immunological studi have been performed Also in the other reported cases of this syndrome no imm nological tests were carried out

In our patient while the serum Ig leve were within the normal limits the number B and—to a larger extent—of T lymphocyte evaluated by the E and EA rosette formi cells respectively was definitely below t normal range (see Table 1) Our results su gest that a complex impairment of the i munologic function involving not only t humoral but also the cellular immune r sponse can be found in the Schwartz Jam syndrome

REFERENCES

1 Beighton P The Schwartz syndrome in South Africa *Clin Genet* 4 548 1973

Boyum A Separation of lymphocytes from blood and bone marrow *Scand J Clin Lab Invest 21* Suppl 97 1 1968
Burgio G R Severi F Ugazio A G & Vaccaro R In *Tema di Deficien e della immunita specifica Proc 38th Congr Ital Soc Pediatr* Minerva Medica Torino 1976 p 51
Hallberg T Gurner B W & Coombs R R A Opsonic adherence of sensitized ox red cells to human lymphocytes as measured by rosette formation *Int Arch Allergy 44* 550 1973
Jondal M Holm G & Wigzell H Markers of human T and B lymphocytes I A large population of lymphocytes forming non immune rosettes with sheep red blood cells *J Exp Med 136* 207 1972
6 Kirschner B S & Pachman L M IgA deficiency and recurrent pneumonia in the Schwartz Jampel syndrome *J Pediatr 88* 1060 1976

Submitted Febr 2 1978
Accepted May 8 1978

(L P) Clinica Pediatrica
V le A Doria
Città Universitaria
95125 Catania
Italy

Acta Pædiatr Scand 68 137–144 1979

REVIEW ARTICLE

INTERACTIONS OF NUTRITION INFECTION AND IMMUNE RESPONSE

Immunocompetence in Nutritional Deficiency Methodological Considerations and Intervention Strategies

R K CHANDRA

From the Department of Pediatrics Memorial University of Newfoundland St John s Newfoundland Canada

ABSTRACT Chandra R K (Department of Pediatrics Memorial University of Newfoundland St John s Newfoundland Canada) Interactions of nutrition infection and immune response Immunocompetence in nutritional deficiency methodological considerations and intervention strategies Acta Paediatr Scand 68 137 1979.—Clinical and epidemiologic data point to a causal interrelationship between nutritional deficiency and infectious illness Both are major contributors to childhood morbidity and mortality particularly in under privileged population groups Energy protein undernutriti n and deficiencies of iron folates and pyridoxine depress a variety of immunity functions Delayed hypersensitivity and number of T lymphocytes are consistently reduced In small for-gestation low birth weight infants cell mediated immunity may remain depressed for several years B lymphocytes immunoglobulin levels and antibody responses are generally normal but secretory IgA antibody is reduced Serum complement components are low and there is evidence of in vivo consumption of complement C3 Neutrophil phagocytosis of bacteria and fungi is intact but the next step of intracellular killing is impaired. There are changes also in the production of lysozyme and interferon Infection per se results in nutrient losses either actual or by sequestration and produces immunosuppression The correction of postnatal nutritional deficits and/or infection is associated with reversal of immunological functions to normal The interplay of nutrition immunity and infection and its biological implications are described

KEY WORDS Malnutrition immunity lymphocyte function neutrophils complement system infection

The stark spectrum of over 500 million human beings suffering from the multiple erosive effects of undernutrition is a rude eye opener for any serious student of global health problems Malnutrition affects each and every function of the body The most prominent association of nutritional deficiency is with infection The impression is widely held by clinicians that undernutrition increases the frequency and severity of infectious illness At the same time a sick child frequently has no desire to eat may have impaired absorption and has greater metabolic needs thereby worsening his nutritional status. Thus infection exacerbates malnutrition and vice versa In this paper recent data on the mechanisms of interactions between nutrition immunity and infection is reviewed

EPIDEMIOLOGY

Many field surveys have documented a higher frequency of infections particularly gastroenteritis and respiratory disease in the under nourished Prophylactic or therapeutic supplementation of diet is associated with a significant reduction in morbidity These observations have been summarized by Scrimshaw et al (35) Among a group of construction and plantation workers in Indonesia acute and

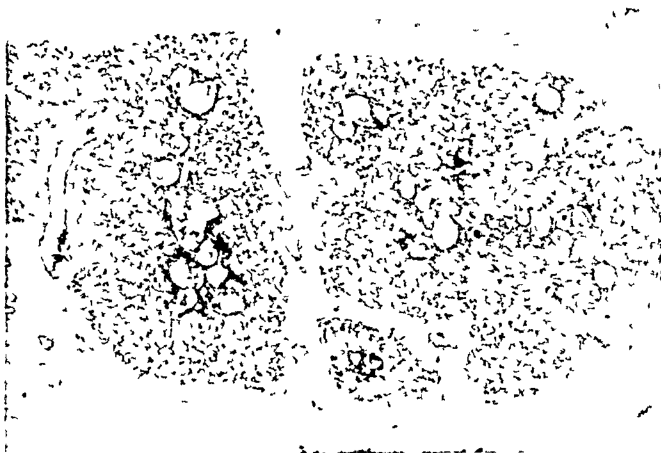


Fig 1 Thymus in severe energy protein undernutrition Note depletion of lymphocytes and crowding of Hassall corpuscles some of which are degenerating

chronic infections were more prevalent in iron deficient anemic individuals than in non anemic controls (1) The effect of iron supplementation on infection related morbidity is variable (12) In moderately severe malnutrition infection is a common primary or contributory cause of death The Pan American Health Organization study of mortality patterns in Central and South America revealed the presence of undernutrition in 57% of the children who died under 5 years of age many of them of terminal infection (34) There is a higher frequency of iron deficiency in patients with chronic mucocutaneous candidiasis (29) and in those with recurrent herpes labialis (23) Human data is strongly supported by the almost invariable heightened susceptibility to infectious challenge in laboratory animals (19 35)

It is essential to point out some of the pitfalls and limitations of the available epidemiologic information on the incidence of infection in malnutrition and vice versa Most of the studies have conducted one point analysis and there was no longitudinal follow up Moreover the common occurrence of infection and nutritional deficiency does not necessarily imply a causal relation but both may have been the result of the same environmental factors e g poverty

MORPHOLOGICAL CHANGES

In moderately severe malnutrition involution of the thymus is a consistent finding (19 40) The lymphocytes are fewer in number and Hassall corpuscles may be degenerated (Fig 1) In lymph nodes and the spleen the

Table I *Immunoglobulin containing plasma cells in the jejunum*

Values are expressed as number per 6 μm × 500 μm tissue Mean ± S D is given

Group	IgA	IgM	IgG
Malnourished	67±17	27±9	5±2
Healthy	93±23	17±5	3±1

thymus dependent areas paracortical and periarteriolar regions respectively are depleted of small lymphocytes (19) In a few patients with advanced malnutrition germinal follicles are also small and few There is little information on the gut associated lymphoid tissues Tonsils are shrunken (3 40) and there are fewer IgA producing cells in the jejunal submucosa (Table I) (14) These alterations in organ size and structure are observed both in acquired postnatal malnutrition (19 40) as well as in intrauterine growth retardation (17 32) Comparable changes can be induced in experimental animals deprived of total energy intake proteins vitamin B_6 or iron

The pathogenesis of such changes in the organs of the immune system is complex Restricted proliferation of cells reduced protein synthesis changes in many hormones endotoxemia and chalones may all be responsible Elevated concentration of free cortisol has been documented in malnourished humans and in deprived experimental animals It is certain that the additional stress of associated infection contributes to structural alterations in the lymphoid tissues in undernutrition

IMMUNOCOMPETENCE

Cell mediated immunity and T lymphocytes

Failure to mount *delayed cutaneous hypersensitivity* reactions in malnutrition has been known for many decades (28) A variety of skin test antigens have been employed including tuberculin purified protein derivative Candida trichophyton mumps streptokinase streptodornase dinitrochlorobenzene and dinitrofluorobenzene (3 33 40) In some studies recovery from nutritional deficiency was associated with the ability to show positive skin responses Impaired delayed hypersensitivity can be the result of thymic dysfunction decreased macrophage and lymphocyte migration to the local site and reduction in nonspecific cutaneous reactivity either singly or in combination It is probable that the first two factors are the more important ones in malnutrition

Depression of cellular immunity in nutritional deficiency has been suggested also by abnormalities in several *in vitro* correlates For example rosette forming *T lymphocytes* in the peripheral blood are almost invariably reduced in proportion (Fig 2) (5 13 25) and absolute number and promptly recover after short term nutritional therapy often before any obvious clinical or biochemical changes (19) In addition to an actual reduction in the number of T lymphocytes it has been suggested that cytophilic inhibitors of rosetting with sheep erythrocytes may be at work (15) Among others IgE (16) and alpha fetoprotein (20) have been reported to be elevated in some malnourished infants and both can inhibit the rosetting process The unique ability of T cells to respond mitotically to the plant lectin

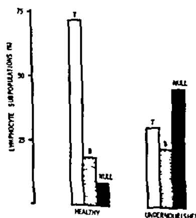


Fig 2 Lymphocyte subpopulations in the peripheral blood of children with energy protein undernutrition and in controls

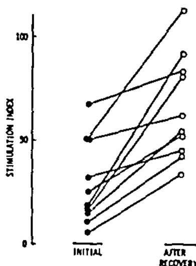


Fig 3 Lymphocyte stimulation response in 10 children with energy protein undernutrition before and after nutritional recovery Lymphocytes isolated from the peripheral blood were cultured in the presence of phytohemagglutin Stimulation index represents the ratio of counts per minute in PHA containing culture/counts in buffer containing culture

phytohemagglutinin has been assessed in malnourished groups and found to be reduced (3 5 33 40) This reduction is somewhat greater than might be explained on the basis of fewer T lymphocytes

The changes in *mitogen induced lymphocyte responses* in malnutrition are completely reversed to normal on dietary supplementation (5) (Fig 3) It is important to note that lymphocyte proliferation is reduced even in marginal undernutrition though not consistently (Fig 4) Besides cellular factors plasma inhibitors of lymphocyte DNA synthesis have been reported in kwashiorkor and marasmus (15 31) In many such studies the influence of associated infection has been impossible to rule out

Alterations in lymphocyte subpopulations and in the *in vivo* and *in vitro* delayed hypersensitivity responses have been noted not only in acquired postnatal energy protein malnutrition but also in small for gestation low birth weight infants (11 23) and in deficiencies of isolated nutrients such as iron (7) folates (27) and vitamin B_6

In nutritional deficiency there is a proportionate increase in the number of *null cells* which do not bear the conventional surface markers of T or B lymphocytes (Fig 2) The functional attributes of null cells are not entirely clear but preliminary information suggests that they can exert cytotoxic and suppressor effects (13) Further studies on defining the function of these cells are required

B lymphocytes immunoglobulins and antibodies

Immunoglobulins and specific antibodies are produced by plasma cells which represent the maturational end stage of B lymphocytes In malnutrition the proportion and number of circulating *B cells* is generally normal (Fig 2) rarely it may be decreased or increased Serum levels of all the major classes of *immunoglobulins* are often high (Table 2) this is considered to be the result of repeated infections experienced by undernourished individuals We have postulated that recurrent protracted infections of the gastrointestinal and respiratory tract occur partly as a result of impaired mucosal immunity (8) in nutritional deficiency and that such infections as well as constant immunological stimulation by the absorbed macromolecules (food antigens products of gut bacterial flora etc) (9) contribute to the polyclonal hyperglobulinemia Rarely a young infant with severe energy

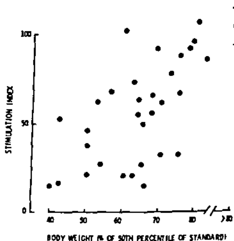


Fig 4 Lymphocyte stimulation related to deficit in body weight The mean±1 S D of values obtained in nutritionally healthy individuals is shown as a bar

Table 2 *Serum immunoglobulin levels*

Group	IgG (mg/100 ml)	IgA (mg/100 ml)	IgM (mg/100 ml)	IgD (mg/100 ml)	IgE (U/ml)
Energy protein undernutrition					
With gross infection	2 360±654	268±56	188±43	16 5±7 1	360±110
With demonstrable parasites*	2 145±721	309±79	244±57	29 3±8 9	2 865±581
Without obvious infection or infestation	625±207ᵃ	87±23	81±25	3 1±1 9	427±129
Well nourished					
With gross infection	2 895±256	220±38	159±33	11 1±5 3	56± 37
With demonstrable parasites	1 435±216	180±53	136±47	7 6±2 9	2 410±736
Without obvious infection or infestation	1 080±19 ᶠ	110±29	88±21	1 9±0 9	36± 21

Values are expressed as mean ± S D
* Ascaris and/or hookworm
Plasma half life of IgG 9 6 days
ᵃ Plasma half life of IgG 31 3 days
Plasma half life of IgG 11 1 days
ᶠ Plasma half life of IgG 19 5 days

From Chandra & Newberne (19)

protein deficiency may have low levels of IgG (3) and other immunoglobulins This is particularly observed in low birth weight infants who experience a profound and prolonged hypoimmunoglobulinemia (11) There is paucity of information on the metabolic turnover rates of immunoglobulins in malnutrition The presence of associated infection accelerates catabolism whereas noninfected children with low serum IgG have a prolonged plasma half life of IgG (Table 2)

Isohemagglutinin titres are normal Elsewhere we have summarized the published data on the effect of nutritional status on *antibody response* to natural infection or to administered microbial and chemical antigens (16 19 21) The difficulties of interpreting most of this information are recognized because of the extreme variations in study protocols In particular failure to administer a potent antigen or to exclude concomitant infection need emphasis In general antibody response to most antigens is adequate in the majority of undernourished individuals Contrarily some particulate antigens e g heterologous red blood cells (10) and *Salmonella typhi* (3) may elicit a reduced response

In kwashiorkor and marasmus *secretory IgA* concentrations in nasopharyngeal secretions tears and saliva are low (8 39 42) and out of proportion to the slight reduction in protein levels in general It is possible that this is the result of reduced synthesis of IgA by the fewer IgA plasma cells in the submucosae (Table 1) or decreased synthesis of secretory component by the atrophied epithelia or both We have observed reduced secretory IgA antibody response to viral vaccines in a group of children suffering from energy protein malnutrition (8) More studies are required in this important area of host defense

Antigen nonspecific protective factors

Complement components are vital elements of amplifying the immune response by promoting chemotaxis immunoconglutination adherence opsonization and microbial lysis Malnutrition causes a reduction in the serum levels of almost all complement proteins (6 38) The changes are more profound in patients with obvious infection and are reversible on dietary supplementation Reduced synthesis as well as increased breakdown have been suggested as the underlying mechanisms Increased consumption is evidenced by the presence of complement C3 degradation products and high titres of immunoconglutinin (6)

Opsonic function of plasma is comparable in healthy and undernourished subjects if tested at more than 10% concentration At lower concentrations of plasma however there is

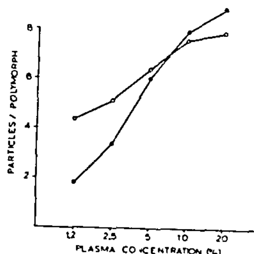


Fig 5 Opsonic function of plasma Bacteria and polymorphonuclear leukocytes were incubated in the presence of plasma of malnourished or well nourished children The number of phagocytosed particles was counted in 100 cells

a significant difference between the two groups (Fig 5) This may be relevant and limit the rate of phagocytosis in tissue spaces where complement components and their opsonic factors are present in concentrations much lower than in serum

The function of *polymorphonuclear leukocytes* has been evaluated in several groups of undernourished children The ability to ingest particles is intact but the killing of phagocytosed bacteria is impaired (Fig 6) (36 37) In an occasional patient virtually no reduction in the count of viable intracellular bacteria is observed paralleling findings in inherited granulocytopathies However the microbicidal defect in malnutrition is reversed completely when the individual's nutritional status is restored to normal (19 37) Similar alterations of phagocyte function are documented in iron deficiency (4 7 30) and in fetal growth retardation (11) An important methodologic consideration needs mention The ratio of the number of bacteria to the number of phagocytes is critical At higher ratios the bactericidal defect of neutrophils from malnourished subjects becomes prominent although it is distinctly demonstrable even at lower ratios of bacteria to phagocytes (Fig 6)

Chemotactic migration of polymorphs is fairly normal except in individuals with infection (22)

Many other nonspecific protective factors including *interferon* and *lysozyme* are altered in nutritional deficiency. These have been reviewed elsewhere (19 35)

EFFECTS OF INFECTION

Infection itself can produce significant changes in nutritional status and immune function The extent of such alterations determined by the severity and duration illness nature of the microbe and the hea status of the individual prior to infection fectious disease particularly febrile illness creases tissue catabolism and urinary los (2) reduces appetite and food intake a may decrease absorption from the gut or ac ally result in protein losing gastroenteropatl The combined result of all these processes i further deterioration of nutritional status is well known that measles increases losses nutrients in the feces and the urine lowe serum albumin concentration (Fig 7) and m precipitate edema in a child with margi undernutrition Infection *per se* depress

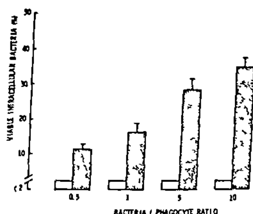


Fig 6 Bactericidal capacity of polymorphonuclear leukc cytes in energy protein undernutrition related to the rati of the number of bacteria to phagocytes *Staphylococcu aureus* was the organism used and viable intracellula bacteria were counted at 20 and 140 min of culture anc expressed as a percentage ratio Open columns represen healthy controls and the shaded columns undernourishec children Means and S E M of 15 subjects in each group are shown

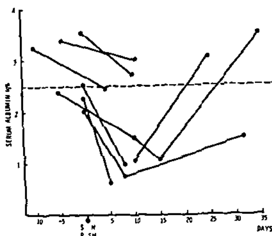


Fig 7 Effect of measles on nutritional status measured by serum albumin concentration In all patients a significant drop in albumin level occurred In those with low normal level before the infection there was a more pronounced fall in serum albumin concentration often associated with the appearance of dependent edema Recovery occurred several weeks following medical nutritional management From Chandra & Newberne (19)

many aspects of immunity function possibly by the invasion of lymphoid tissues altering the proportion of lymphocyte subpopulations especially suppressor cells changing hormonal levels endotoxemia and by the elaboration of acute phase reactant alpha globulins and chalones Measles in the pre exanthematous phase and for several weeks after the appearance of the rash impairs mitogen induced lymphocyte stimulation even though the numbers of T lymphocytes remain unchanged (19) These changes in cellular immunity have prognostic significance (24) Lymphocyte changes also have been described in acute malaria (26) Similarly infection related granulocyte dysfunction may adversely influence the outcome of the infectious process (41)

CONCLUDING REMARKS

The inextricably intertwined problems of undernutrition and infection defy easy analysis in terms of the underlying mechanisms of interactions Recent studies have revealed impaired function of a variety of host protective factors It is possible that the clinical problem of repeated and severe infections in malnourished persons is the result of a summation of variable individual abnormalities in several key cellular and humoral immunological functions These changes in immunocompetence may play a critical role in the effectiveness of immunization in the susceptibility to septicaemic spread of infection and in the production of immunopathological disease Infection *per se* can result in significant deficits in immunity function and in nutrition At the same time it must be recognized that nutritional status is intricately tied to other health factors and that the roots of malnutrition are largely economic To tackle this major world affliction we must adopt a portfolio approach of intervention at several levels including socioeconomic development nutrition education promotion of breast feeding agricultural output immunizations safe water supply and environmental sanitation (18) The task looks formidable but that is no reason for not trying

REFERENCES

1 Basta S S & Churchill A *Iron deficiency anemia and the productivity of adult males in Indonesia* Staff Working Paper 175 World Bank Washington DC 1974
2 Beisel W R Metabolic response to infection *Ann Rev Med* 26 9 1975
3 Chandra R K Immunocompetence in undernutrition *J Pediatr 81* 1184 1972
4 Chandra R K Reduced bactericidal capacity of polymorphs in iron deficiency *Arch Dis Child 48* 863 1973
5 Chandra R K Rosette forming T lymphocytes and cell mediated immunity in malnutrition *Br Med J 3* 608 1974
6 Chandra R K Serum complement and immunoconglutinin in malnutrition *Arch Dis Child 50* .?5 1975
7 Chandra R K Impaired immunocompetence associated with iron deficiency *J Pediatr 86* 899 1975
8 Chandra R K Reduced serum and secretory antibody response to live attenuated measles and poliovirus vaccines in malnourished children *Br Med J II* 583 1975
9 Chandra R K Food antibodies in malnutrition *Arch Dis Child 50* 53? 1975

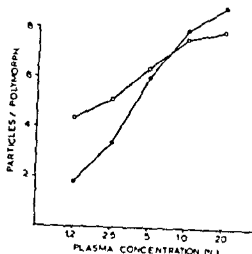


Fig 5 Opsonic function of plasma Bacteria and polymorphonuclear leukocytes were incubated in the presence of plasma of malnourished or well nourished children The number of phagocytosed particles was counted in 100 cells

a significant difference between the two groups (Fig 5) This may be relevant and limit the rate of phagocytosis in tissue spaces where complement components and their opsonic factors are present in concentrations much lower than in serum

The function of *polymorphonuclear leukocytes* has been evaluated in several groups of undernourished children The ability to ingest particles is intact but the killing of phagocytosed bacteria is impaired (Fig 6) (36 37) In an occasional patient virtually no reduction in the count of viable intracellular bacteria is observed paralleling findings in inherited granulocytopathies However the microbicidal defect in malnutrition is reversed completely when the individual's nutritional status is restored to normal (19 37) Similar alterations of phagocyte function are documented in iron deficiency (4 7 30) and in fetal growth retardation (11) An important methodologic consideration needs mention The ratio of the number of bacteria to the number of phagocytes is critical At higher ratios the bactericidal defect of neutrophils from malnourished subjects becomes prominent although it is distinctly demonstrable even at lower ratios of bacteria to phagocytes (Fig 6)

Chemotactic migration of polymorphs is fairly normal except in individuals with infection (22)

Many other nonspecific protective factors including *interferon* and *lysozyme* are altered in nutritional deficiency These have been reviewed elsewhere (19 35)

EFFECTS OF INFECTION

Infection itself can produce significant changes in nutritional status and immunity function The extent of such alterations is determined by the severity and duration of illness nature of the microbe and the health status of the individual prior to infection Infectious disease particularly febrile illness increases tissue catabolism and urinary losses (2) reduces appetite and food intake and may decrease absorption from the gut or actually result in protein losing gastroenteropathy The combined result of all these processes is a further deterioration of nutritional status It is well known that measles increases losses of nutrients in the feces and the urine lowers serum albumin concentration (Fig 7) and may precipitate edema in a child with marginal undernutrition Infection *per se* depresses

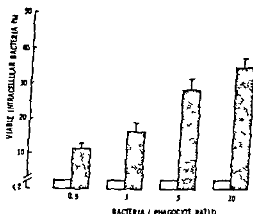


Fig 6 Bactericidal capacity of polymorphonuclear leukocytes in energy protein undernutrition related to the ratio of the number of bacteria to phagocytes *Staphylococcus aureus* was the organism used and viable intracellular bacteria were counted at 20 and 140 min of culture and expressed as a percentage ratio Open columns represent healthy controls and the shaded columns undernourished children Means and S E M of 15 subjects in each group are shown

NEW BOOKS RECEIVED

S S Gellis & B M Kagan *Current Pediatric Therapy* vol 8 879 pp W B Saunders Philadelphia London Toronto 1978 £ 3 00 ISBN 0-7?16-4089-3

J B Hanshaw & J A Dudgeon *Viral Diseases of the Fetus and Newborn* (Vol XVII in the series Major Problems in Clinical Pediatrics) 347 pp illus W B Saunders Co Philadelphia London Toronto 1978 £14 00 ISBN 0 7 16-4500-3

A Moragas A Ballabriga & M T Vidal *Atlas of Neonatal Histopathology* ?30 pp illus W B Saunders Co Philadelphia London Toronto 1977 £43 75 ISBN 0-7?16-654? X

Jean de Grouchy & Catherine Turleau *Clinical Atlas of Human Chromosomes* 319 pp illus John Wiley & Sons New York Chichester Brisbane Toronto 1977 £ 4 70 ISBN 0-471-01704 3

Bernbeck & Sinios *Neuro-Orthopedic Screening in Infancy* Schedules Examinations and Findings 1 0 pp illus Urban & Schwarzenberg Munchen 1978 DM 8 – £7 70 $13 50 ISBN 3 541 70 31 1

Derrick B Jelliffe & E F Patrice Jelliffe *Human Milk in the Modern World* 500 pp illus Oxford University Press 1978 £15 00 ISBN 0-19 64919 1

David J Boullin (ed) *Serotonin in Mental Abnormalities* 316 pp John Wiley & Sons Chichester New York Brisbane Toronto 1978 £13 50 ISBN 0-471 99501-0

L Gedda & P Parisi (eds) *Auxology Human Growth in Health and Disorder* (Sereno Symposium no 13) 319 pp illus Academic Press Inc London 1978 £11 0 $? 00 ISBN 0-1? 79050-?

G Chiumello & Z Laron (eds) *Recent Progress in Pediatric Endocrinology* Proceedings of the Sereno Symposia Vol 1? 325 pp Academic Press London New York San Francisco 1977 £10 00 $19 50 ISBN 0-1 173 50-9

R Bernbeck & A Sinios *Neuro-Orthopedic Screening in Infancy* Schedules Examinations und Findings 1 0 pp illus Urban & Schwarzenberg Baltimore Munchen 1978 US$13 50 ISBN 3 541 70?31 1

P A Zorab (ed) *Scoliosis* 38 pp illus Academic Press London New York San Francisco 1977 £7 80 ISBN 0-1 781850-

Harry Shirkey *Pediatric Drug Handbook* 198 pp W B Saunders Company Philadelphia London Toronto 1977 No price given ISBN 0-7 16-8 47

Atherine E Snow & Charles A Ferguson (eds) *Talking to Children* 369 pp Cambridge University Press Combridge London New York Melbourne 1977 £8 00 ISBN 0-5 1 1318 5

James F Bosma (ed) *Symposium on Development of the Basic anim* U S Department of Health Education and Welfare 00 pp illus U S Government Printing Office Washington D C 040 Stock Number (NIH) 77 989 £13 5

Noeman A Beischer & Eric V Mackay *Obstetrics and the Newborn* 53 pp illus Holt Saunders Pty Ltd 1976 £8 50 ISBN 003 900051-6

O G Eiben (ed) *Growth and Development* Physique Symposium on Human Biology Balatonfüred Tihany 508 pp illus Akademiai Kiadó Budapest 1977 $30 00 ISBN 963 05 1355 2

Zvi Laron Petah Tikva (ed) *Medical Aspects of Balance of Diabetes in Juveniles In* Pediatric and Adolescent Endocrinology Vol 2 60 pp illus S Karger Basel Munchen Paris London New York Sydney 1977 DM 98 – US$37 75 ISBN 3 8055 2388-2

Hans Weidinger (ed) *Labor Inhibition Betamimetic Drugs in Obstetrics* ?62 pp illus Gustav Fischer Verlag Stuttgart New York 1977 DM 4? – ISBN 3-437 10449-7

D B Jelliffe & J P Stanfield (eds) *Diseases of Children in the Subtropics and Tropics* ?nd ed 1049 pp illus Edward Arnold (Publishers) Ltd 1978 £25 00 ISBN 0-7131-4?77-4

The Fetus and Birth Ciba Foundation Symposium 47 481 pp Elsevier Excerpta Medica/North Holland 1977 No price given ISBN 0-444 15?55 5

Schroder & Brandt *Longitudinalstudie uber Wachstum und Entwicklung von fruh und reifeborenen Kindern von der Geburt bis zum 6 Lebensjahr* 56 Seiten illus DM 13 – Westdeutscher Verlag GmbH 1977 ISBN 3 531-0?691 7

J Borms & M Hebbelink (eds) *Pediatric Work Physiology* Vol II *In* Medicine and Sport Series (ed E Jokl & H Hebbelink) 178 pp illus S Karger Basel Munchen Paris London New York Sydney 1978 US$39 00 ISBN 3 8055 ?866-3

M H Klaus & A A Fanaroff *Das Risiko-Neugeborne* Diagnostik und Therapie 344 pp illus Gustav Fischer Verlag Stuttgart New York 1978 DM 78 – ISBN 3-437 105 6-4

P Dasen B Inhelder M Lavallee & J Retschitzki *Naissance de l'intelligence che l enfant baoule de Cote d'Ivoire* 3 4 pp illus Hans Huber Bern Stuttgart Vienne 1978 DM 48 – ISBN 3-456-80659-0

E Rossi (ed) *Neuere Aspekte der Kinderkardiologie In* Padiatrische Fortbildungskurse fur die Praxis No 47 108 pp illus S Karger Basel Munchen Paris London New York Sydney 1978 US$? 00 ISBN 3 8055 ?865-5

A K K Kreutner & D R Hollingsworth *Adolescent Obstetrics and Gynecology* 658 pp Year Book Medical Publishers Inc Chicago London 1978 $50 50 ISBN 0-8151 5 00-0

Claude A Frazier *Sniff Sniff Al er gee* 36 pp illus Johnny Reads Inc St Petersburg Florida 1978 $? 65 ISBN 0-91081 ?4 1

J L Melnick (ed) *Progress in Medical Virology* Vol 4 4 pp illus S Karger Basel Munchen Paris London New York Sydney 1978 US$54 00 ISBN 3 8055 810-8

H Homewood Nixon (ed) *Operative Surgery* 3rd edition 489 pp illus Butterworths London Baston 1978 £4? 50 ISBN 0-407-00635-6

10 Chandra R K Antibody formation in first and second generation offspring of nutritionally deprived rats *Science 190* 289 1975
11 Chandra R K Fetal malnutrition and postnatal immunocompetence *Am J Dis Child 129* 450 1975
12 Chandra R K Iron and immunocompetence *Nutr Rev 34* 129 1976
13 Chandra R K Lymphocyte subpopulations in malnutrition Cytotoxic and suppressor cells *Pediatrics 59* 423 1977
14 Chandra R K Nutrition dietary factors and immunology of the gut In *Tripartite Symposium on Gastroenterology* City University London 1977
15 Chandra R K Cell mediated immunity in fetally and postnatally malnourished children from India and Newfoundland *In* R M Suskind (ed) *Malnutrition and the immune response* Raven Press New York 1977 p 111
16 Chandra R K Immunoglobulins and antibody response in malnutrition *In* R M Suskind (ed) *Malnutrition and the immune response* Raven Press New York 1977 p 155
17 Chandra R K Ontogenetic development of the immune system and effects of fetal growth retardation *J Perinat Med 7* 1979 In press
18 Chandra R K Nutritional deficiency and susceptibility to infection Epidemiological clinical data immune responses and effects of breast feeding transfer factor and levimasole *Bull WHO 56* 1978 In press
19 Chandra R K & Newberne P M *Nutrition immunity and infection Mechanisms of interactions* Plenum New York 1977
20 Chandra R K & Bhujwala R A Elevated α feto protein and impaired immune response in malnutrition *Int Arch Allergy Appl Immunol 53* 80 1977
21 Chandra R K Chakraburty S & Chandra S Malnutrition humoral immunity and infection *Indian J Pediatr 43* 159 1976
22 Chandra R K Chandra S & Ghai O P Chemotaxis random mobility and mobilization of polymorphonuclear leucocytes in malnutrition *J Clin Pathol 29* 224 1976
23 Chandra R K Au B Woodford G & Hyam P Iron status immunocompetence and susceptibility to infection In *Ciba Foundation Symposium on Iron Metabolism* Elsevier Amsterdam 1977 p 249
24 Coovadia H M Wesley A Brain P Henderson L G Hallett A R & Vos G H Immunoparesis and outcome in measles *Lancet I* 619 1977
25 Ferguson A S Lawlor G J Jr Neumann C G Oh W & Stiehm E R Decreased rosette forming lymphocytes in malnutrition and intrauterine growth retardation *J Pediatr 85* 717 1974
26 Greenwood B M Oduloju A J & Stratton D Lymphocyte changes in acute malaria *Trans Roy Soc Trop Med Hyg 71* 408 1978
27 Gross R L Reid J V O Newberne P M Burgess B Marston R & Hift W Depressed cell mediated immunity in megaloblastic anemia due to folic acid deficiency *Am J Clin Nutr 28* 225 1975
28 Harland P S E G Tuberculin reactions in malnourished children *Lancet I* 719 1965
29 Higgs J M & Wells R S Chronic mucocutaneous candidiasis Associated abnormalities of iron metabolism *Br J Dermatol* Suppl 8 88 1973
30 MacDougall L G Anderson R McNab G M Katz J Immune response in iron-deficient children impaired cellular defense mechanisms with altered humoral components *J Pediatr 86* 833 1975
31 Moore D L Heyworth D & Brown J PHA induced lymphocyte transformation in leucocyte cultures from malarious malnourished and control Gambian children *Clin Exp Immunol 17* 647 1974
32 Naeye R L Blanc W & Paul C Effects of maternal nutrition on the human fetus *Pediatrics* 494 1973
33 Neumann C G Lawlor C G Stiehm E R Swedseid M E Newton C Herbert J Ammann A J & Jacob M Immunological responses in malnourished children *Am J Clin Nutr 28* 89 1975
34 Puffer P R & Serrano C V *Patterns of mortality in childhood* Pan American Health Organization Washington D C 1974
35 Scrimshaw N S Taylor C E & Gordon J E *Interactions of nutrition and infection* World Health Organization Geneva 1968
36 Selvaraj R J & Bhat K S Metabolic and bactericidal activities of leukocytes in protein-calorie malnutrition *Am J Clin Nutr 25* 166 1972
37 Seth V & Chandra R K Opsonic activity phagocytosis and intracellular bactericidal capacity of polymorphs in undernutrition *Arch Dis Child 47* 1972
38 Sirisinha S Suskind R Edelman R Charupatana C & Olson R E Complement and C3 proactivator levels in children with protein calorie malnutrition *Lancet I* 1016 1973
39 Sirisinha S Suskind R Edelman R Asvapaka C & Olson R E Secretory and serum IgA children with protein calorie malnutrition *Pediatrics 55* 166 1975
40 Smythe P M Breton Stiles G G Grace H Mafoyane A Schonland M Coovadia H M Loening W E K Parent M A & Vos G H Thymolymphatic atrophy and depression of cell mediated immunity in protein calorie malnutrition *Lancet II* 939 1971
41 Solberg C O & Hellum K M Neutrophil granulocyte function in bacterial infections *Lancet II* 1972
42 Watson R Reyes M A & McMurray D N Influence of malnutrition on the concentration of Ig lysozyme amylase and aminopeptidase in children's tears *Proc Soc Exp Biol Med 157* 215 1978

Submitted Aug 17 1978
Accepted Aug 31 1978

(R K C) Janeway Child Health Centre
St John s Newfoundland A1A 1R8
Canada

BOOK REVIEWS

M Siegel *The clinical management of muscle disease practical manual of diagnosis and treatment* William Heinemann London 1977 167 pp illus £3 75 ISBN 0-433 30 75 5

As stated in the preface this monograph has been written as a compendium on the general problems of muscle diseases with the practical aspects of diagnosis and care being stressed As the author is an orthopaedic surgeon it is natural that the emphasis is directed toward physiatric and orthopaedic therapeutic techniques The author s personal experience is reflected in the valuable chapters dealing with the postural pathomechanics following weakness and contractures in Duchenne dystrophics as well as the survey of surgical treatment of hip ankle and foot deformities The difficult problem of scoliosis is also dealt with

The reader looking for a description of the new congenital myopathies will be disappointed The section on genetics is not written with the same authority as those about practical treatment The illustrations consisting of photographic documentation of patients are not of a very high quality There are numerous spelling errors in the text In spite of its weaknesses the volume is worth to be read also by members of a habilitation team the management of orthopaedic problems in muscular dystrophy being to a large extent neglected in neuropaediatric textbooks

Bo Hellstrom

W Plenert & J Hermann *Anamien im Kindesalter* Reihe Moderne Padiatrie VEB Georg Thieme Leipzig 1977 147 pp illus DM 35

For an Anglo-American oriented Swede it was kind of an adventure to read this little book in paperback from GDR It shows very clearly that paediatric haematology is well developed in Jena where the authors live and work Further it is obvious that the authors have had access to all pertinent and recent literature even from abroad and by these means and by their own clinical experience and studies they have been able to compile a comprehensive and up-to-date presentation of childhood anaemias Mostly the story is told in a clear and straight forward way which makes the reading easy even for those who are not very familiar with German Unnecessary and confusing deviations which used to be common in German scientific publications have been avoided There are of course statements that could be disputed but they are rare and of minor importance

[illegible]

R G Williams & C R Tucker *Echocardiographic diagnosis of congenital heart disease* 352 pp illus Little Brown and Company Boston 1977 $19 95 ISBN 0-316-94351 7

The use of echocardiography in paediatric cardiology has increased rapidly during the last years and it is therefore natural that handbooks and monographs on this subject are now appearing The title of the present book is a little misleading since it covers echocardiography in various parts of paediatric cardiology not only congenital heart disease

The book is based on the experience of the Boston group since 1972 It is thus founded on a large patient series The book is divided into four parts the first dealing with the basic technique the second with normal echocardiography including anatomy and the third and fourth part discuss the diagnosis of acyanotic and cyanotic heart diseases Each chapter is presented in a logical way presenting anatomy examination technique diagnostic features divided into definite suggestive and supporting features and ending with discussion of pitfalls and differential diagnoses Each chapter contains several clea illustrative echocardiograms explaining schematic drawings and a list of pertinent references As a whole th presentation is made in a very clear didactic way

My main objection to this presentation concerns th limitations of the ultrasound method These are to som extent discussed in the sections named pitfalls It could however have been valuable with an additional discussion about the limitations and pitfalls set by ultrasoun physics such as the problem of lateral resolution th beam width the angulation problem with echo dropou etc The statement that the diagnosis of a ventricul septal defect can be made by echocardiography seems me dangerous and is certainly a controversial subject

The main abnormal echocardiographic findings a discussed in a proper way but information of how reliab these signs are would have increased the value of t book

With such a new technique as echocardiography d ferences in opinion and interpretation cannot be avoid and must be accepted The future will increase our e perience and tell us more about the reliability of the va ous abnormal findings As a whole I think it is importa always to interpret the echocardiogram in relation to the other diagnostic information available

This book can be recommended to all working with echocardiography in paediatric cardiology One should read it bearing in mind that this is a new technique and that the implication of some echocardiographic abnormalities described needs confirmation in larger materials in order to verify the diagnostic usefulness and particularly the reliability of the method

Nils Rune Lundstrom

E Rossi (ed) *Moderne Endoscopie im Kindesalter In* Padiatrische Fortbildungskurse fur die Praxis No 46 122 pp illus S Karger Basel Munchen Paris London New York Sydney 1977 US$24 50 ISBN 3 8055 2799 3

John O Forfar & Gavin C Arneil (eds) *Textbook of Paediatrics 2nd edition Vol 1 and 2* 27?? pp illus Churchill Livingstone Edinburgh London and New York 1978 £50 00 ISBN 0-443 01848-0

ANNOUNCEMENTS

XXth CONGRESS OF THE CZECHOSLOVAK PAEDIATRIC SOCIETY

The XXth Congress of the Czechoslovak Paediatric Society *with International Participation will take place in* Bratislava October 3–5 1979 For further information write to the Congress Office Slovak Medical Soc Mickiewiczova 18 88322 Bratislava Czechoslovakia

INTERNATIONAL MEETING ON PATHOPHYSIOLOGY OF PUBERTY

An international meeting on Pathophysiology of Puberty will be held in Bologna Italy June 21–22 1979 For further information please write to Professor Emanuele Cacciari Clinica Pediatrica dell Universita Via Massarenti 40138 Bologna Italy

THE EIGHTEENTH ANNUAL LEO G RIGLER LECTURE

The 18th Annual Leo G Rigler Lecture and Convention on Pediatric Radiology—sponsored by the Tel Aviv University Sackler School of Medicine section of diagnostic radiology and the municipal—government medical center Tel Aviv/Yaffo departments of diagnostic radiology is to be held at the Accadia hotel Herzlia on Sea on May 8th to 10th 1979 The Rigler lecturer will be John A K patrick jr M D professor and radiologist in-c Children s Hospital Medical Center Boston Mass further information kindly contact professor S Sch Ichilov Hospital Tel Aviv Israel

THE INTERNATIONAL WORKSHOP ON THE AT RISK INFANT

The International Workshop on the At Risk Infant will take place in *Tel Aviv (Israel) July 25–27 30–31 1979* the International Year of the Child For further information write to the secretary Shaul Harel M D P O *16271 Tel Aviv Israel*

BOOK REVIEWS

J M Siegel *The clinical management of muscle disease A practical manual of diagnosis and treatment* William Heinemann London 1977 16? pp illus £3 75 ISBN 0-433 30?75 5

As stated in the preface this monograph has been written as a compendium on the general problems of muscle dis ea es with the practical aspects of diagnosis and care being stressed As the author is an orthopaedic surgeon it is natural that the emphasis is directed toward physiatric and orthopaedic therapeutic techniques The author s personal experience is reflected in the valuable chapters dealing with the postural pathomechanics following weak ness and contractures in Duchenne dystrophics as well as the survey of surgical treatment of hip ankle and foot deformities The difficult problem of scoliosis is also dealt with

The reader looking for a description of the new con genital myopathies will be disappointed The section on genetics is not written with the same authority as those about practical treatment The illustrations consisting of photographic documentation of patients are not of a very high quality There are numerous spelling errors in the text In spite of its weaknesses the volume is worth to be read also by members of a habilitation team the management of orthopaedic problems in muscular dys trophy being to a large extent neglected in neuropaediatric textbooks

Bo Hellstrom

W Plenert & J Hermann *Anamien im Kindesalter* Reihe Moderne Padiatrie VEB Georg Thieme Leipzig 1977 14 pp illus DM 35

For an Anglo-American oriented Swede it was kind of an adventure to read this little book in paperback from GDR It shows very clearly that paediatric haematology is well developed in Jena where the authors live and work Further it is obvious that the authors have had access to all pertinent and recent literature even from abroad and by these means and by their own clinical experience and stud es they have been able to compile a comprehensive and up-to-date presentation of childhood anaemias Most ly the story is told in a clear and straight forward way which makes the reading easy even for those who are not very familiar with German Unnecessary and confusing deviations which used to be common in German scientific publications have been avoided There are of course statements that could be di puted but they are rare and of minor importance

Stig Sjolin

R G Williams & C R Tucker *Echocardiographic diagnosis of congenital heart disease* 352 pp illus Little Brown and Company Boston 1977 $19 95 ISBN 0-316-94351 7

The use of echocardiography in paediatric cardiology has increased rapidly during the last years and it is therefore natural that handbooks and monographs on this subject are now appearing The title of the present book is a little misleading since it covers echocardiography in various parts of paediatric cardiology not only congenital heart *disease*

The book is based on the experience of the Boston group since 1972 It is thus founded on a large patient series The book is divided into four parts the first dealing with the basic technique the second with normal echo-cardiography including anatomy and the third and fourth parts discuss the diagnosis of acyanotic and cyanotic heart diseases Each chapter is presented in a logical way presenting anatomy examination technique diagnostic features divided into definite suggestive and supporting features and ending with discussion of pitfalls and dif ferential diagnoses Each chapter contains several clear illustrative echocardiograms explaining schematic draw ings and a list of pertinent references As a whole the presentation is made in a very clear didactic way

My main obje tion to this presentation concerns the limitations of the ultrasound method These are to some extent discussed in the sections named pitfalls It could however have been valuable with an additional discus sion about the limitations and pitfalls set by ultrasound physics such as the problem of lateral resolution the beam width the angulation problem with echo dropouts etc The statement that the diagnosis of a ventricular septal defect can be made by echocardiography seems to me dangerous and is certainly a controversial subject

The main abnormal echocardiographic findings are discussed in a proper way but information of how reliable these signs are would have increased the value of the book

With such a new technique as echocardiography dif ferences in opinion and interpretation cannot be avoided and must be accepted The future will increase our ex perience and tell us more about the reliability of the vari ous abnormal findings As a whole I think it is important always to interpret the echocardiogram in relation to all the other diagnostic information available

This book can be recommended to all working with echocardiography in paediatric cardiology One should read it bearing in mind that this is a new technique and that the implication of some echocardiographic abnor malities described needs confirmation in larger materials in order to verify the diagnostic usefulness and partic ularly the reliability of the method

Nils Rune Lundstrom

ACKNOWLEDGEMENT

The Editorial Board of Acta Paediatrica Scandinavica wishes to express its sincere gratitude to the following persons outside the Advisory Board who have acted as referees during the past year. The standard of the journal depends to a very large extent on the skill and interest of these reviewers.

Ø. Aagenæs
K. Aas
H. Ahlström
S. Almqvist
G. E. Andersen
H. J. Andersen
K. E. Andersson
Anita Aperia
M. Arborelius
Birgitta Aronsen
S. Aronson
N. O. Berg
T. Berg
A. Bergstrand
Å. Bertler
J. Bjure
J. I. Björkhem
B. Björksten
G. Bjorlin
G. Bodegård
M. Bohman
P. Bolme
S. Boréus
P. Brandtzaeg
O. Broberger
R. Brodersen
C. Brook
P. Canlorbe
B. Cavell
J. L. Chaussain
S. Cronberg
G. Dahlqvist
J. Ditzel
O. Eeg Olofsson
T. Ehrenpreis
M. Ekberg
O. Eklöf
B. Eriksson
Margareta Eriksson
S. Eriksson
G. Fex
H. Feychting
A. Forsgren
Marianne Forslund
T. Foucard
S. P. Fällström
Ingrid Gamstorp
S. Garwicz
J. Gentz
D. Grant
A. Gustafsson
K. H. Gustafsson
L. Gustafsson
E. Guttler
B. Hadorn
S. Hagberg
S. Halvorsen
L. Hambraeus
L. Å. Hanson
A. Hansson
P. Henriksson
Y. Hofvander
J. Holmgren
R. Holst
A. Huch
Gunnel Huldt
N. Høiby
S. Ivarsson
B. Ivemark
B. Brock Jacobsen
S. Jacobsson
Birgitta Jalling
J. O. Jeppsson
J. C. Job
V. Jodal
G. Johansson
T. Johnson
R. Jonsell
B. Jonson
C. O. Kindmark
N. I. Kjellman
I. Kjellmer
D. Knorr
E. Kock
O. Koskimies
U. Krause
A. Kreuger
B. Kallen
L. Kohler
R. Lagercrantz
B. Lagerkvist
B. Landtman
A. Larsson
Anna Brita Laurell
G. Laurell
L. Lidgren
G. Lindgren
G. Lindstedt
J. Lindsten
F. Linell
J. Ludvigsson
N. R. Lundström
E. Lycke
Margret Lynch
B. Magnusson
J. Malmquist
P. Malvaux
G. Meeuwisse
A. Melander
J. Melchior
M. Michaelsson
F. Mitelman
D. Moore
Erna Möller
Inga Marie Nilsson
K. O. Nilsson
I. Nylander
E. Odeblad
L. Okmian
R. Olegård
Ö. Ouchterlony
K. Palmén
B. Persson
O. Pettay
L. Philipson
A. Prader
J. Rapola
A. Rausing
P. Riis
M. Ritzén
H. Rorsman
L. Sann
J. C. L. Shaw
B. Siesjö
A. Siimes
P. C. Sizonenko
S. Sjöblad
A. Sjöholm
U. Smith
B. Sporrong
R. Steendijk
Birgitta Strandvik
T. Sveger
L. Svennerholm
N. Svenningsen
Eivor Svenonius
R. Sörnäs
L. Tejler
W. Teller
G. Theander
C. Thoren
K. A. Thulin
N. G. Toremalm
R. Tunell
L. Van den Brande
C. de Verdier
L. Victorin
J. Vidnes
E. Vinnars
T. Wadström
G. Wallgren
Sigbritt Werner
O. Westphal
G. Wettrell
L. Wide
J. Winberg
S. Winblad
O. Wolff
L. Wranne
M. Zachmann
L. Åhström
P. A. Öckerman

Acta Pædiatr Scand 68 149–154 1979

LONG TERM PROGNOSIS OF INFANTS WITH IDIOPATHIC RESPIRATORY DISTRESS SYNDROME

Follow up Studies in Infants Surviving after the Introduction of Continuous Positive Airway Pressure

JENS KAMPER and JØRN MØLLER

From the Department of Neonatology Rigshospitalet University of Copenhagen Copenhagen Denmark

ABSTRACT Kamper J & Møller J (Department of Neonatology Rigshospitalet University of Copenhagen Denmark) Long term prognosis of infants with idiopathic respiratory distress syndrome Follow up studies in infants after the introduction of Continuous Positive Airway Pressure Acta Paediatr Scand 68 149 1979 —Fifty one children surviving IRDS with CPAP alone or CPAP and IPPV were studied at the age of 2 5 to 4 0 years One child had developed tetraplegia and mental retardation and 6 children were speech retarded Correlation with perinatal events showed that this group of children had a significantly lower gestational age and birth weight a lower Apgar score and a higher P_{CO_2} prior to ventilatory treatment than the remainder Re examination by age 4 0 to 5 0 years showed persistent handicaps in only four of the seven children

KEY WORDS Newborn infants respiratory distress syndrome artificial respiration neurological findings

Ventilatory support of infants with idiopathic respiratory distress syndrome (IRDS) is today a well established procedure In a relatively short span of years technique and methods have been developed which have improved the early prognosis of this serious condition remarkably A particularly important milestone was the introduction of the continuous positive airway pressure (CPAP) (10 24) which significantly simplified the treatment lessening the risks of dangerous complications Publications dealing with the long term prognosis from this period are scarce (1) In the following we therefore will present our follow up results in 51 children who survived IRDS after CPAP had begun to be used

MATERIALS AND METHODS

The neonatal department of Rigshospitalet has employed mechanical ventilation since 1966 ( ) CPAP was adopted in August 1971 and during the following 20 months a total of 94 infants with IRDS received ventilatory support either with CPAP intermittent positive pressure ventilation (IPPV) or both (Fig 1) Fifty five infants survived (58 5%) but 4 were lost to follow up leaving a total of 51 survivors to participate

IRDS was diagnosed when the infants developed progressive respiratory failure with tachypnoea grunting recessions and increasing oxygen dependency and the characteristic chest X ray configuration could be demonstrated (21) Neonatal and therapeutical characteristics of the 51 survivors are indicated in Table 1 Twenty nine (57%) infants were born after pregnancies complicated by bleeding episodes pre-eclampsia placental insufficiency rhesus immunization or maternal diabetes mellitus and 21 (41%) were delivered by caesarean section or in irregular presentations Seven infants had an Apgar score equal to 3 or lower after 5 minutes or did not cry during this period Six infants had a birth weight lower than 1 501 g and 6 infants were small for-dates having birth weights below the 10th centile (3) Twenty four infants (47%) were born in other hospitals and transferred as emergency cases Thirty were boys and 21 girls

Umbilical artery catheters were inserted when the infants required more than 40% oxygen for the purpose of following the arterial P_{O_2} and acid–base status and to provide a parenteral route for fluid administration Infants admitted in gross respiratory failure were not catheterized till after the ventilatory support had been initiated however In 6 survivors the arterial catheterization failed Whenever umbilical arterial catheters were not available oxygen saturation and acid-base parameters were obtained on blood drawn by heel stabs The Pa and acid

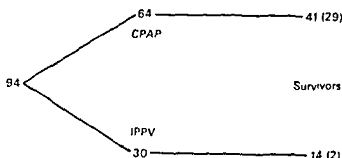


Fig 1 94 infants distributed according to primary mode of ventilatory support Infants surviving with only one mode of ventilatory support are indicated in parentheses

base analyses were performed using the Radiometer BMS II

Parenteral fluid therapy was started on the first day of life and gastric tube feeding with mother s milk or milk substitute (Nan®) was initiated on the second Accurate information concerning milk intake could be obtained in 44 infants who received an average of 502 ml milk per kilo birth weight during the first week of life Records of body weight at the end of the first week were available in 41 infants

CPAP was administered *ad modum* Gregory et al (10) As shown in Fig 1 a total of 64 infants could be treated initially with CPAP indicated by P_{O_2} values below 6 66–7 98 kPa (50–60 mm) and/or general cyanosis combined with hypercapnia and acidosis in spite of breathing pure oxygen through a close fitting face mask These conditions had previously been chosen as indications of ventilator therapy in our department (2 14)

CPAP was mostly administered via naso tracheal tube (18) The so-called Gregory box (10) was tried out in 8 infants 6 of whom survived The box was abandoned because of difficulties with the neck seal which caused ulcerations in 2 cases

Twenty nine out of 64 infants survived with CPAP only while 12 survivors also received IPPV because of progressive respiratory insufficiency in spite of 12–14 cm CPAP and 100% oxygen Eighteen survivors received treatment with CPAP because of a low Pa_{O_2} (mean 5 72) and 18 because of combined general cyanosis hypercapnia (mean P_{CO_2} 7 85 kPa) and acidosis (mean pH 7 23)

As indicated in Fig 1 23 infants died—2 during CPAP treatment the remainder while on ventilator Autopsy showed massive intracranial haemorrhage in 13 and pulmonary haemorrhage in 1 bronchopneumonia in 3 a isolated pulmonary fibroplasia in 1 infant In 4 cases death was ascribed to intractable atelectasis

Mean birth weight was relatively high in infants surviving with CPAP only (2 320±660 g) when compared w *survivors* who also needed IPPV (1896±785 g $p<0\ 0$ and *all infants* needing IPPV later (1 798±681 g $p<0\ 01$ No significant differences between the above mentione groups were found in mean values of gestational age Pa_{O_2} pH and P_{CO_2} prior to or in age at initiation CPAP

Ventilator treatment

Ventilatory techniques and methods used have been published previously (2) The indication for ventilator treatment in these series was general cyanosis and highly in sufficient respiratory movements (often apnoea) in spite 100% inspiratory oxygen Thirty infants were ventilate but only 14 survived (Fig 1)

Preventilatory acid-base values were obtained in 6 survivors (mean pH 7 15 mean P_{CO_2} 10 37 kPa) Twelve of 14 survivors were weaned off the respirator usin CPAP

Autopsy performed in all fatal cases except one showe intracranial haemorrhage in 6 significant diffuse pulmonary haemorrhage in 1 and bronchopneumonia in 1 in fant In the remainder death was ascribed to massive diffuse atelectasis

Ophthalmoscopic examinations

These were performed on the ward at regular intervals an repeated in case of suspect findings and in infants with birth weight below 2 001 g when at least 2 months old

Follow up procedure

Forty four survivors were examined at our follow up clinic while 6 had to be examined at home One of these who had emigrated was examined by a local physician Another child who also lives abroad participated by questionnaire only With these two exceptions all children were examined by one of the authors (J M) Four survivors did not participate in the follow up but 2 of these were judged normal at routine controls by age 3 years

Table 1 *Neonatal and therapeutical characteristics of 51 infants surviving with ventilatory support*

	Range	Mean	(Mean)
Birth weight (g)	1 250–3 550	2 197	(2 446)
Gestational age (weeks)	28–39	34 2	(34 8)
Age ventilatory support begun (h)	4–55	25	(35)
Duration of ventilatory support (h)	3–633	101	(144)
Duration of oxygen treatment ≥70% (h)	3–355	72	(211)
Duration of oxygen treatment any conc (h)	67–1 800	263	(425)

Mean values are indicated in parentheses for 75 infants surviving IRDS with the aid of IPPV from the preceding 5 year period

Table 2 *Result of neurological and psychological examination in 7 IRDS survivors with doubtful or certain handicaps at first follow up*

	First follow up			Last follow up			
Patient	Age (yr)	Linguistic test	DDST	Age (yr)	Leiter performance test (IQ)	Binet intelligence test (IQ)	Major neurological and psychological findings
VM	9	Abnormal	Abnormal	4 6	91	–	Tetraplegia
TJN	3 25	Abnormal	Normal	4 0	98	–	Dysphasia epilepsia
KH	75	Abnormal	Normal	4 5	104	77	Minimal brain dysfunction
TM	3 3	Abnormal	Normal	5 0	107	90	Dysphasia
KAC	75	Abnormal	Normal	4 0	111	97	Dysphasia?
PSL	2 5	Abnormal	Normal	4 1	116	100	0
BM	5	Abnormal	Normal	4 0	116	108	0

The age at follow up ranged from 5 to 4 years with a mean of 3

A questionnaire was forwarded to the parents to allow them to complete in advance anamnestic details of the child and for comparison the next younger or older sibling if any On the day of the follow up the questionnaires were scrutinized the children were given a physical and neurological examination and height weight and head circumference were measured Fifty children were evaluated with the revised Denver developmental screening test (DDST) (8 9) which has proven both sensitive and specific with a high degree of agreement with more comprehensive developmental and intelligence tests Vocabulary and linguistics were evaluated further with a Danish linguistic test (5) which is standardized for children between 3 0 and 6 5 years Reference values for children below 3 0 years of age were calculated by extrapolation Seven children who presented with major neurological abnormalities or abnormal linguistic performance were admitted to paediatric neurologists or psychologists for more detailed evaluation which usually included Binet s intelligence test (Danish revision 1943) and always Leiter s performance test

Children seen at Rigshospitalet had chest X rays taken in the antero-posterior and lateral projections The radiographs were evaluated blindly by 2 radiologists (H S and L J)

RESULTS

Growth

Compared to values predicted from recent Swedish growth curves (6) the mean weight of the survivors was 0 4 kg below mean for age while mean height did not differ The mean head circumference was 1 0 cm above mean No child had a head circumference larger than ±2 S D of the curves however and none was suspected of developing hydrocephalus

Development

Early motor development was estimated from parental dating of the first step taken without support One child who had developed a tetraplegia did not walk by the age of 2 75 years The remainder walked on an average by age 14 4 months Thirty two survivors were compared with their siblings The ability to walk alone was found on an average to be retarded by 1 9 months ($p<0\ 01$)

As to speech development 40% of the IRDS survivors had developed considerably more slowly than their siblings which is significant ($\chi^2=9\ 4$ $p<0\ 01$)

Neurological and psychological examination

A total of 7 IRDS survivors presented with doubtful or certain handicaps by age 2 5 to 4 years as indicated in Table 2 Only 4 of these had remaining moderate to severe handicaps when re-evaluated at the age of 4 to 5 years however Strabismus which was found in 10 children and febrile convulsions occurring in 4 were not considered handicaps None developed retrolental fibroplasia

The 7 IRDS survivors with abnormal linguistic performance including the child with cerebral palsy were compared with the remainder with regard to a number of neonatal parameters employing Student s t test as shown in Table 3 The statistical analysis showed that this group of children was not

Table 3 *Neonatal and therapeutical parameters in 43 children with normal and 7 with abnorm linguistic performance (Bo Ege Linguistic Test)*
NS=Not Significant

	Normal		Abnormal		Probability
	N	Mean±1 S D	N	Mean±1 S D	
Gestational age (weeks)	43	34 0±2 4	7	31 8±2 2	<0 02
Birth weight (g)	43	2 277±600	7	1 734±193	<0 05
Apgar score 1 min	40	7 5±2 5	7	4 2±1 9	<0 005
Apgar score 5 min	37	8 9±1 5	7	7 8±1 4	NS
P_{CO_2} before ventilatory support (kPa)	34	8 1±1 9	5	10 9±3 2	<0 01
pH before ventilatory support	34	7 21±0 09	5	7 12±0 06	NS
Age ventilatory support begun (h)	43	26±16	7	18±17	NS
Duration of ventilatory support (h)	43	94±97	7	152±153	NS
Intake of milk (ml/kg birth weight/1 wk)	36	495±213	7	512±154	NS
Weight loss (g/kg birth weight/1 wk)	35	79±44	6	87±36	NS

only more asphyctic at delivery smaller and more premature but also more asphyctic prior to ventilatory treatment than the other group The mode of ventilatory treatment was of no prognostic significance e g the child with cerebral palsy survived with CPAP only

Cardio pulmonary examination

Ten IRDS survivors had been admitted to hospital at least once because of lower respiratory tract infections No children suffered from laryngeal stenosis and none presented with dyspnoea or cyanosis One child presented with a patent ductus arteriosus which was successfully closed 6 months later

The X ray examination revealed minor changes in 10 IRDS survivors the predominating abnormality being discrete parenchymal line shadows None had emphysema or cor pulmonale

DISCUSSION

During the last decade ventilatory support of newborn infants with respiratory insufficiency mainly IRDS has come into common use Controlled studies have reported increased survival rates with ventilator treatment (19 22) and a number of follow up studies has encouraged the development further in that the majority of these high risk infants has been shown to develop normally (4 7 1 12 13 14 17 20 23) These studies have al shown that some survivors may develop s nificant handicaps e g cerebral palsy hyd cephalus intellectual deficiency and retrole tal as well as pulmonary fibroplasia Resu from different centres are difficult to comp due to differences in composition of materia i e number of referrals and very small p matures and probably in a large number therapeutical details Comparisons from p riod to period within the same centre se more adequate although it must be emphasiz that various improvements in nursery ro tines monitoring etc may influence the ou come Compared with the preceding 5 ye period (14) the survival rate in infants ven lated for IRDS at our centre increased fro 38 6 to 58 5% (and later to nearly 80% (15 At the same time the prevalence of neurolo cal and mental sequelae decreased from 17 8% and the type and extent of handicaps b came more moderate As an increased numb of small prematures has survived (Table these changes strongly suggest that a conside able improvement in the outlook of our ven lated IRDS survivors has taken place Mo survivors in this study have been treated e clusively or partially with CPAP which thought to have both modified the ventilator treatment as a whole as indicated in Table

and contributed significantly to improvements in both short (15) and long term prognosis

Although only 4 survivors displayed lasting handicaps more infants are thought to have suffered from cerebral depression during the perinatal period as 32 survivors who could be compared with siblings seemed retarded as to their early motor and speech development Furthermore 7 survivors at the time of the first follow up were classified as speech retarded at the linguistic test which is 3 or 4 more than predicted This early speech retardation was significantly related not only to low birth weight and gestational age but also to low Apgar score at 1 minute and hypercapnia prior to ventilatory support Similarly Hof & Weisser (12) found a significant relation between retarded psychomotor development and preventilatory hypercapnia The possible risk of early hyponutrition has been stressed by Stahlman and co workers (23) who speculated whether psychological deficiencies in childhood could be ascribed to neonatal starvation during mechanical ventilation Our previous follow up study (14) did in fact give support to this theory as survivors with psychomotor or mental retardation had been supplied with significantly less milk per kg birth weight than the remainder during the first week of life In contrast speech retardation in the present series was not related to low milk intake (or weight loss) during the first week This may be due to the fact that the average milk intake has increased considerably i e from 305 to 502 ml/kg birth weight

Thus further improvements in long term prognosis given adequate nutrition may depend largely on prevention of *preventilatory* asphyxia for instance by increased use of cardiotochographia and by earlier initiation of ventilatory support (16)

ACKNOWLEDGEMENTS

The study has been supported by Statens Åndssvage forsorgs forskningsudvalg (grant no 3051/7 177) The authors thank Dr Henrik Schioeler and Dr Lars Jacobsen for evaluating the chest radiographs

REFERENCES

1 Ahlstrom H Jonson B & Svenningsen N W Continuous positive airways pressure treatment by a face chamber in idiopathic respiratory distress syndrome *Arch Dis Child 51* 13 1976

2 Cooke R Lunding M Lomholt N F Yssing M Zachau-Christiansen B & Friis-Hansen B Respiratory failure in the newborn The techniques and results of intermittent positive pressure ventilation *Acta Pædiatr Scand 56* 498 1967

3 Davies P A Robinson R J Scopes J W Tizard J P M & Wigglesworth J S Birth weight/gestational age charts In *Clinics in developmental medicine* nos *44/45 Medical care of newborn babies* Spastics International Medical Publications London 1972 W Heinemann Medical Books Ltd Oxford J B Lippincott Co Philadelphia p 373

4 Dinwiddie R Mellor D H Donaldson S H C Tunstall M E & Russel F Quality of survival after artificial ventilation of the newborn *Arch Dis Child 49* 703 1974

5 Ege B Sproglig test I Sprogaldertest *Tale og Stemme 1* 21 1969

6 Engstrom I Karlberg P Klackenberg G Klackenberg Larsson I Lichtenstein H Svennberg I & Taranger J Growth diagrams for height weight and head circumference from birth to the age of 18 years *Lakartidningen 70* 2952 1973

7 Fitzhardinge P M Pape K Arstikaitis M Boyle M Ashby S Rowley A Netley C & Swyer P R Mechanical ventilation of infants of less than 1 501 gm birth weight Health growth and neurological sequelae *J Pediatr 88* 531 1976

8 Frankenburg W K & Dodds J B The Denver developmental screening test *J Pediatr 71* 181 1967

9 Frankenburg W K Goldstein A D & Camp B W The revised Denver developmental screening test Its accuracy as a screening instrument *J Pediatr* 79 988 1971

10 Gregory G A Kitterman J A Phibbs R H Tooley W H & Hamilton W K Treatment of the idiopathic respiratory distress syndrome with continuous positive airway pressure *N Engl J Med* 284 1333 1971

11 Harrod J R L Heureux P Wangensteen O D & Hunt C E Long term follow up of severe respiratory distress syndrome treated with IPPB *J Pediatr 84* 277 1974

12 Hof R & Weisser K Spatfolgen nach intermittierender Uberdruckbeatmung bei schwerem idiopatischem respiratory distress syndrome *Schwei Med Wochenschr* 99 1853 1969

13 Johnson J D Malachowski N C Grobstein R Welsh D Daily W J R & Sunshine P Prognosis of children surviving with the aid of mechanical ventilation in the newborn period *J Pediatr 84* 272 1974

14 Kamper J Long term prognosis of infants with severe idiopathic respiratory distress syndrome I Neurological and mental outcome *Acta Pædiatr Scand* 67 61 1978

15 Kamper J Bækgaard P Eeitersen B Marstrand Christiansen P Tygstrup I & Friis Hansen B Respiratory distress syndrome of the newborn treated with continuous positive airway pressure *Ugeskr Læg 135* 2207 1973
16 Krouskopp R W Brown E G & Sweet A Y The early use of continuous positive airway pressure in the treatment of idiopathic respiratory distress syndrome *J Pediatr 87* 263 1975
17 Marriage K J & Davies P A Neurological sequelae in children surviving mechanical ventilation in the neonatal period *Arch Dis Child 52* 176 1977
18 Marstrand Christiansen P Kamper J Peitersen B Bækgaard P & Friis Hansen B Technique and method of treatment of respiratory distress syndrome with continuous positive airway pressure (CPAP) *Ugeskr Læg 135* 2212 1973
19 Murdock A I Linsao L McC Reid M Sutton M D Tilak K S Ulan O A & Swyer P R Mechanical ventilation in the respiratory distress syndrome A controlled trial *Arch Dis Child 45* 624 1970
20 Outerbridge E W Ramsay M & Stern L Developmental follow up of survivors of neonatal respiratory failure *Crit Care Med 2* 23 1974
21 Peterson H G & Pendleton M E Contrasting roentgenographic patterns of the hyaline membrane and the fetal aspiration syndromes *Am J Roentgenol 74* 800 1955
22 Reid D H S Tunstall M E & Mitchell R G A controlled trial of artificial respiration in the respiratory distress syndrome of the newborn *Lancet 1* 532 1967
23 Stahlman M Hedwall G Dolanski E Faxelius G Burko H & Kirk V A six year follow up of clinical hyaline membrane disease *Pediatr Clin N Am 20* 433 1973
24 Wung J T Stark R L Hegyi T Driscoll J M & James L S CDP A major breakthrough *Pediatrics 58* 783 1976

Submitted Febr 3 1978
Accepted June 25 1978

(J K) Department of Paediatrics
Odense Sygehus
DK 5000 Odense
Denmark

Acta Pædiatr Scand 68 155–159 1979

A CLINICAL AND NEUROPHYSIOLOGICAL INVESTIGATION OF A DANISH KINDRED WITH HETEROZYGOUS FAMILIAL HYPOBETALIPOPROTEINEMIA

G E ANDERSEN W TROJABORG and H C LOU

From the Neonatal Department and the Department of Neurophysiology Rigshospitalet Copenhagen Denmark

ABSTRACT Andersen G E Trojaborg W and Lou H C (Neonatal Department and Department of Neurophysiology Rigshospitalet Copenhagen Denmark) A clinical and neurophysiological investigation of a Danish kindred with heterozygous familial hypobeta lipoproteinemia Acta Paediatr Scand 68 155 1979 —A three-generation transmission of under five percentile values for serum low density lipoprotein and low density lipoprotein cholesterol typical of heterozygous familial hypobetalipoproteinemia was demonstrated in a Danish family Slight clinical signs of CNS abnormality were found in 4 of the 8 subjects with heterozygous familial hypobetalipoproteinemia but did not resemble the neurological findings in abetalipoproteinemia nor in the previously described patients with familial hypobetal poproteinemia There were no signs of myelin dysfunction in the central nervous system as judged from the normal latency of visual and somatosensory evoked potentials

KEY WORDS Cholesterol triglyceride lipoproteins genetics myelin EEG cortical evoked potentials

Familial hypobetalipoproteinemia (FHBL) is a genetic disorder which differs from classical abetalipoproteinemia (ABL) by having an abnormally low yet immunochemically identifiable serum LDL in the propositus and at least one first degree relative in the absence of diseases to which hypobetalipoproteinemia may be secondary (trauma infections fat malabsorption hyperthyroidism hepatic necrosis severe anemia anti apo B myeloma protein production) An autosomal dominant mode of inheritance is most likely (1) FHBL has been described in a heterozygous and homozygous form The heterozygous condition is normally asymptomatic there is however data which suggests that the low serum LDL C levels may deter the development of coronary heart disease leading to a longer than average life expectancy (2) Homozygotes are biochemically indistinguishable from patients with ABL having no apolipoprotein B nor LDL but do not seem to have the same serious central and peripheral neuromuscular degeneration typical of ABL (3 4 5 6) The cause of the low serum LDL concentration is obscure Levy et al (7) studied the metabolism of radioiodinated LDL and found a 3 fold lower synthetic rate of LDL in FHBL heterozygotes compared to normals Furthermore Sigurdsson et al (8) have found a decreased synthetic rate of VLDL apo B and LDL apo B in heterozygotes Serum LDL is supposed to be a vehicle for substances such as cholesterol linoleic acid vitamin A and E probably all essential for the metabolism of myelin (9 10 11) Therefore in FHBL subjects with very low levels of serum LDL signs of impaired myelination might be expected In 6 of the 57 heterozygotes so far recorded have

Abbreviations

FHBL=familial hypobetalipoproteinemia ABL=abetalipoproteinemia T C=total cholesterol VLDL-C=very low density lipoprotein cholesterol LDL-C=low density lipoprotein cholesterol HDL-C=high density lipoprotein cholesterol TG=triglyceride VEP=visual evoked potentials SEP=somatosensory evoked potentials

Table 1 *Serum lipids and lipoproteins in the individual subjects*

Kindred	Age (years)	T C (mmol/l)	VLDL C (mmol/l)	VLDL (EID) (mg/100 ml)	LDL C (mmol/l)	LDL (EID) (mg/100 ml)
I 2	75	3.58	0.52	44	1.67	318
II 1	46	3.47	0.47	42	1.75	337
III 1	19	2.87	0.21	18	0.70	171
III 2	17	3.24	0.25	23	0.84	179
III 3	14	4.34	0.06	5	1.78	377
III 4	12	3.94	0.15	15	1.11	229
III 5	10	5.22	0.12	16	2.70	496
III 6	8	3.53	0.08	8	1.31	261
III 7	7	3.97	0.08	0	1.18	44
III 8	5	4.34	0.12	12	2.63	450
III 9	3	4.54	0.04	8	2.63	682
III 10	1 7/12	3.37	0.20	20	1.33	274
III 11	At birth	1.65	0.06	-	0.39	-

neurological symptoms been described (12, 13, 14, 15). In 3 of the 6, however, it is not even certain that the neuromuscular abnormalities were related to low levels of serum LDL.

Since the disorder has not earlier been recorded in Scandinavia and since the neurological symptoms in FHBL are still very scant and inconsistent, we here present the clinical and neurophysiological findings in a Danish family with heterozygous FHBL.

Table 2 *Neurological and neurophysiological findings in the individual subjects*

Kindred	Age (years)	Clinical findings	EEG	SEP and VEP
I 2	75	Babinski sign bilaterally. Impaired vibration sense (absent on lower extremities)	Normal	Normal
II 1	46	Babinski sign (right). Slight finger dysdiadokokinesia	Normal	Normal
III 1	19	None	Normal	Normal
III 2	17	Atypical plantar reflexes. Increased extensor tone. Slight choreoathetosis. Slight constructional dyspraxia	3–4 Hz activity, predominantly in temporal regions	Normal
III 3	14	None	*Normal*	*Normal*
III 4	12	None	Normal	Normal
III 5	10	None	At rest normal. Generalized 4–5 Hz paroxysmal activity with spikes evoked by photic stimulation	Normal latency. Amplitudes 125% increased at stimulation of both median nerves
III 6	8	Prominent associated movements	*Normal*	*Normal*
III 7	7	Increased extensor tone. *Prominent* associated movements. Choreoathetosis and finger dyskinesia. Slight constructional dyspraxia	Focal spike activity in left temporal region. Normal background activity	Normal
III 8	5	None	*Normal*	*Normal*
III 9	3	None	Normal	Normal
III 10	1 7/12	None	Normal	Normal
III 11	At birth	None	Normal	[illegible]

DL C (mmol/l)	TG (mmol/l)	Diagnoses
39	1 40	FHBL
5	1 73	FHBL
96	0 73	FHBL
15	0 79	FHBL
50	0 51	Normal
68	0 71	FHBL
40	0 77	Normal
14	0 65	FHBL
71	0 71	FHBL
59	0 88	Normal
87	0 73	Normal
1 84	0 89	FHBL
1 0	0 30	Normal

MATERIAL

During our screening of 10000 Danish newborns for hyper and hypolipoproteinemia in February 1976 we identified a newborn girl with a very low level of cord serum VLDL–LDL (<5th percentile value for normal Danish newborns (16)) She was born as number 10 of 10 children Pregnancy and delivery was uneventful No drugs had been given and there were no signs of perinatal asphyxia She was born in the 39th week of gestation and weighed 3950 g Apgar score was 10/1 and 5 min The neonatal and subsequent development has been normal All members of her family were investigated and she was herself reinvestigated at age 1?/ years Mean while child number 11 also a girl was born Pregnancy and delivery was normal Her cord serum lipids were also determined

METHODS

Mixed arterial and venous cord blood was obtained after clamping and cutting the umbilical cord within the first 3 min after birth and prior to delivery of the placenta The cord blood was stored at 4 C for no longer than 1? hours before serum was separated (000 r p m 0 min) and the analyses begun Venous blood samples were taken from the family members after a 10–1 hour fast during a period of stable weight and food habits The serum was separated and stored at 4°C and the analyses begun with n 4 hours Serum T-C TG VLDL-C LDL-C were measured by the methods described earl er (17) Lipoproteins were separated in a 40 3 rotor Beckman Type L ultracentrifuge at 10°C at 40000 r p m for 0– hours Tube slicing was used as described earlier (17) Furthermore VLDL and LDL was measured by electroimmunodiffusion (EID) using an ant body raised against apo B as described by Andersen & Gry Nielsen (17)

The clin cal and neurophysiological examination was made by H C L and W T without prior knowledge of the individual's serum lipid and lipoprotein values At the time of the exam nation none of the subjects had been informed of the diagnosis nor were any of them aware of or disabled by neurological symptoms

Visual potentials (VEP) were evoked by flash or pattern reversal and recorded between electrodes placed over the occipital lobes with a reference electrode placed 2 cm in front of vertex during bilateral monocular stimulation as described by Dawson (18 19) Cobb & Morton (?0) and Halliday et al (?1) Somatosensory potentials (SEP) were evoked by stimulating the median nerve at wrist and recorded between an electrode placed over the hand area of the contralateral sensory cortex and one 2 cm in front of vertex as described by Dawson (18 19) and Giblin (?) The leads were connected to EMG amplifiers (DISA 15Col) with a lower limiting frequency of 0 5 Hz and an upper limiting frequency of 1000 Hz (3 dB down) The evoked cortical potentials were recorded on line by electronic averaging of 1 8–?56 responses using a 4 channel signal averager (Nicolet 1074) and was displayed via a X Y writer (HP 7044 A)

RESULTS

The kindred is presented in Fig 1 and the individual serum lipids diagnoses clinical and neurophysiological findings are given in Tables 1 and 2 Three of the children with FHBL (III 2 III 4 and III 7) deserve special attention as they have additional symptoms to the ones outlined in Table 2

III 2 is a 17 year-old girl who for the last three months before the study experienced orthostatic dizziness the cause of which seems to be intermittent hypertension systolic blood pressures being 120–160 and diastolic blood pressures between 80–100 mmHg There were no signs of retinopathy nor cardiac ventricular hypertrophy Renal and adrenal causes of hypertension were ruled out as was coarctation of the aorta Glucose tolerance test was normal Minor neurological and neurophysiological abnormalities were found as described in Table 2

III 4 is a 12 year old boy with diabetes mel

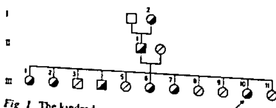


Fig 1 The kindred

Table 1 *Serum lipids and lipoproteins in the individual subjects*

Kindred	Age (years)	T C (mmol/l)	VLDL C (mmol/l)	VLDL (EID) (mg/100 ml)	LDL C (mmol/l)	LDL (EID) (mg/100 ml)
I 2	75	3.58	0.52	44	1.67	318
II 1	46	3.47	0.47	42	1.75	337
III 1	19	2.87	0.21	18	0.70	171
III 2	17	3.24	0.25	23	0.84	169
III 3	14	4.34	0.06	5	1.78	377
III 4	12	3.94	0.15	15	1.11	279
III 5	10	5.22	0.17	16	2.70	496
III 6	8	3.53	0.08	8	1.31	61
III 7	7	3.97	0.08	0	1.18	244
III 8	5	4.34	0.12	12	2.63	480
III 9	3	4.54	0.04	8	2.63	682
III 10	1 7/12	3.37	0.20	20	1.33	274
III 11	At birth	1.65	0.06	–	0.39	–

neurological symptoms been described (12, 13, 14, 15). In 3 of the 6, however, it is not even certain that the neuromuscular abnormalities were related to low levels of serum LDL.

Since the disorder has not earlier been recorded in Scandinavia and since the neurological symptoms in FHBL are still very scanty and inconsistent we here present the clinical and neurophysiological findings in a Danish family with heterozygous FHBL.

Table 2 *Neurological and neurophysiological findings in the individual subjects*

Kindred	Age (years)	Clinical findings	EEG	SEP and VEP
I 2	75	Babinski sign bilaterally. Impaired vibration sense (absent on lower extremities)	Normal	Normal
II 1	46	Babinski sign (right). Slight finger dysdiadokokinesia	Normal	Normal
III 1	19	None	Normal	Normal
III 2	17	Atypical plantar reflexes. Increased extensor tone. Slight choreoathetosis. Slight constructional dyspraxia	3–4 Hz activity, predominantly in temporal regions	Normal
III 3	14	None	Normal	Normal
III 4	12	None	Normal	Normal
III 5	10	None	At rest normal. Generalized 4–5 Hz paroxysmal activity with spikes evoked by photic stimulation	Normal latency. Amplitudes 125% increased at stimulation of both median nerves
III 6	8	Prominent associated movements	Normal	Normal
III 7	7	Increased extensor tone. Prominent associated movements. Choreoathetosis and finger dyskinesia. Slight constructional dyspraxia	Focal spike activity in left temporal region. Normal background activity	Normal
III 8	5	None	Normal	Normal
III 9	3	None	Normal	Normal
III 10	1 7/12	None	Normal	Normal
III 11	At birth	None	Normal	Normal

Cottrill C Glueck C J Leuba V Millet F Puppione D & Brown W V Familial homozygous hypobetalipoproteinemia *Metabolism* 23 779 1974
Salt H B Wolff O H Lloyd J K Fosbrooke A S Cameron A H & Hubble D V On having no beta lipoprotein A syndrome comprising abetalipoproteinemia acanthocytosis and steatorrhea *Lancet II* 3 5 1960
Muller D P R & Lloyd J K The long term management of abetalipoproteinemia a possible role for vitamin E *Arch Dis Child* 52 09 1977
Biemer J J & McCammon R E The genetic relationship of abetalipoproteinemia and hypobetalipoproteinemia A report of the occurrence of both diseases within the same family *J Lab Clin Med* 85 556 1975
Levy R I Langer T Gotto A M & Fredrickson D S Familial hypobetalipoproteinemia a defect in lipoprotein synthesis *Clin Res* 18 539 1970
Sigurdsson G Nicoll A & Lewis B Turnover of apolipoprotein B in two subjects with familial hypobetalipoproteinemia *Metabolism* 26 5 1977
9 Isselbacher K J Scheig R Plotzkin G R & Caulfield J B Congenital beta lipoprotein deficiency An hereditary disorder involving a defect in the absorption and transport of lipids *Medicine (Baltimore)* 43 347 1964
10 Fredrickson D S Levy R I & Lees R S Fat transport in lipoproteins An integrated approach to mechanisms and disorders *N Engl J Med* 276 96 1967
11 Lees R S & Ahrens E H Fat transport in abetalipoproteinemia The effects of repeated infusions of β lipoprotein rich plasma *N Engl J Med* 280 1 61 1969
12 Mars H Lewis L A Robertson A L Butkus A & Williams G H Familial hypo-β lipoproteinemia A genetic disorder of lipid metabolism with nervous system involvement *Am J Med* 46 886 1969
13 Brown B J Lewis L A & Mercer R D Familial hypobetalipoproteinemia Report of a case with psychomotor retardation *Pediatrics* 54 111 1974
14 Tamir I Levtow O Lotan D Lequin C Heldenberg D & Werbin B Further observations on familial hypobetalipoproteinemia *Clin Genet* 9 149 1976
15 Mawatari S Iwashita H & Kuroiwa Y Familial hypo-β lipoproteinemia *J Neurol Sci* 16 93 1972
16 Andersen G E Lous P & Friis Hansen B Screening for hyper and hypolipoproteinemia in 10000 Danish newborns *Acta Paediatr Scand* in press
17 Andersen G E & Gry Nielsen H Neonatal screening for hyperlipoproteinemia Methods for direct estimation of cord serum VLDL LDL *Clin Chim Acta* 66 79 1976
18 Dawson G D Cerebral responses to electrical stimulation of peripheral nerve in man *J Neurol Neurosurg Psychiat* 10 134 1947
19 Dawson G D A summation technique for the detection of small evoked potentials *Electroencephalogr Clin Neurophysiol* 6 65 1954
20 Cobb W A & Morton H B The human electroretinogram in response to high intensity flashes *Electroencephalogr Clin Neurophysiol* 4 547 1952
21 Halliday A M McDonald W I & Mushin J Delayed visual evoked response in optic neuritis *Lancet I* 982 1972
22 Giblin D R Somatosensory evoked potentials in healthy subjects and in patients with lesions of the nervous system *Ann NY Acad Sci* 112 93 1964

Submitted July 3 1978
Accepted Sept 13 1978

(G E A) Neonatal Department GN 50 4
Rigshospitalet
Blegdamsvej 9
DK 2100 Copenhagen
Denmark

litus since the age of 4 Initially he was treated with protamine zinc insulin (Retard Leo®) 20 IU in the morning and 1 600 kcal/day Over the years he has experienced hypoglycemic episodes demanding minor adjustments of the amount of insulin and calories He is at present well regulated on zinc insulin (Monotard Novo®) 18 IU + Semi Lente (Novo®) 10 IU in the morning and 2200 kcal/day His growth and psycho motor development have been normal Neither neurological nor neurophysiological abnormalities were found

III 7 a 7 year old girl with diabetes mellitus since the age of 6 was initially treated with protamine zinc insulin (Retard Leo®) 6 IU in the morning and 1 690 kcal/day She has had diabetic acidosis twice in connection with otitis media After adenoidectomy she is now well regulated on protamine zinc insulin (Retard Leo®) 18 IU + regular insulin (Neutral Leo®) 2 IU in the morning and 2 000 kcal/day Her growth and psychic development have been normal Minor neurological abnormalities were found and her EEG was severely abnormal as described in Table 2

DISCUSSION

In ABL subjects with a total lack of serum apolipoprotein B and LDL the clinical symptoms and signs suggest a combination of neuronal and myelin sheath degeneration In FHBL subjects with very low levels of serum apolipoprotein B and LDL one might therefore expect minor signs of myelin dysfunction Thus far 57 individuals have been reported to have FHBL In only three cases however have neurological abnormalities been described

In a 37 year old woman Mars et al (12) found clumsiness dysesthesia of lower extremities general weakness and hyperactive deep tendon reflexes Babinski sign and ataxia During 1–2 years her condition deteriorated with relapses suggestive of multiple sclerosis Tamir et al (14) found FHBL in a 4 month old boy with microcephaly and infantile spasms Brown et al (13) found FHBL in a 6-month old boy with slow psychomotor development but without seizures

In the present kindred four of the eight individuals with FHBL had slight–moderate clinical signs of CNS abnormality The 75 year old grandmother (I 2) and her 46-year old son (II 1) had abnormal plantar responses suggesting minimal dysfunction of the cortico spinal tracts In the two girls (III 2 and III 7) the findings suggest involvement of both basal ganglia and the cortico spinal tracts These findings however, are not similar to the severe neurological symptoms resembling Friedreich s ataxia in ABL nor can they be related to the neurological findings in the three patients with FHBL previously mentioned

There were no signs of myelin dysfunction in the CNS in any of the eight subjects with FHBL as judged by the normal latency of visual and somatosensory evoked potentials

We therefore conclude that the clinical neurological findings in the present and earlier described individuals with FHBL are either an expression of an exceptional clinical variability or coincidental It is difficult to attribute the findings to one nosological entity i e FHBL Finally the neurophysiological data seem to exclude CNS myelin dysfunction in the FHBL subjects here described

ACKNOWLEDGEMENT

This study was supported by grants from the Danish Heart Foundation the Dagmar Marshall Fund and the Danish Medical Research Council The authors are grateful to the electrophysiological technologists Erna Petersen and Jytte Schmidt for skilful assistence

REFERENCES

1 Herbert P N Gotto A M & Fredrickson D S Familial lipoprotein deficiency In J B Stanbury J B Wyngaarden & D S Fredrickson (eds) *The metabolic basis of inherited disease* McGraw Hill New York 1978 p 544
2 Glueck C J Gartside P Fallat R W Sielski J & Steiner P M Longevity syndromes Familial hypobeta and familial hyperalpha lipoproteinemia *J Lab Clin Med 88* 941 1976

S IgA CHOLERA TOXIN AND ROTAVIRUS ANTIBODY IN HUMAN COLOSTRUM

ALBERTO SIMHON[1,2] ROBERT H YOLKEN[3] and LEONARDO MATA[1]

From the [1]Instituto de Investigaciones en Salud (INISA) Universidad de Costa Rica the [2]Ministerio de Salud San Jose Costa Rica and the [3]Laboratory of Infectious Diseases National Institute of Allergy and Infectious Diseases Bethesda Maryland USA

ABSTRACT Simhon A Yolken R H and Mata L (Instituto de Investigaciones en Salud (INISA) University of Costa Rica San Jose Costa Rica) S-IgA cholera toxin and rotavirus antibody in human colostrum Acta Paediatr Scand 68 161 1979 —S-IgA antibodies against cholera toxin and rotavirus were assayed in 43 colostral samples by means of an enzyme-linked immunosorbent assay (ELISA) All specimens contained specific S-IgA antibodies against both antigens Significant antibody titres to the antigens were demonstrated in almost all colostral samples

KEY WORDS Anti-cholera toxin anti rotavirus colostrum

The aetiology of non specific infantile diarrhoea has been further elucidated by recognition of the pathogenic role of enterotoxigenic *Escherichia coli* (5 12) and rotavirus (1 4) The risk of exposure of infants to these agents particularly in developing countries has stimulated study of the protective effect of breast feeding against their infection and associated diarrhoea

S IgA antibodies to enterotoxins of *Enterobacteriaceae* and *Vibrio cholerae* have been described in human milk by neutralisation assays and the enzyme linked immunosorbent assay (ELISA) (7 15) Also colostra of women from Costa Rica Guatemala and the United States were found to contain considerable amounts of anti rotavirus antibody (21) This report describes a semi quantitative assay of anti-cholera toxin S IgA antibody by ELISA as an alternative to neutralisation techniques Furthermore additional evidence is presented to confirm that colostra from Costa Rican women contain significant levels of anti rotavirus antibody

MATERIALS AND METHODS

Specimens Colostrum from 43 women aged 16 to 30 years from San José Costa Rica were obtained one to 72 hours post partum Mothers were of a medium to-low socio-economic status (approximate per capita yearly income US $70–150) All were at least minimally literate S-IgA was assayed by the ELISA (17) in a modification developed by Yolken et al for rotavirus antigen (0) for labile toxin of *E coli* (19) and for rotavirus antibody assay (21)

ELISA for cholera toxin antibody 2 5 ng of cholera toxin (Schwarz Mann Inc Orangeburg N Y USA) were added to each well of polyvinyl microtitre plates (Cooke Engineering Co Alexandria Virginia USA) precoated with burro anti-cholera toxin antibody Six four fold dilutions of each colostrum sample were added in duplicate wells A conjugate of rabbit anti secretory component (SC) (Dakopatts AS Copenhagen Denmark) and alkaline phosphatase (Sigma Chemical Co St Louis Missouri USA) was then added to each well Addition of p-nitrophenyl phosphate substrate produced a yellow colour the reaction was stopped with 3N NaOH and the colour was read at 400 nm in a Beckman DU ? Spectrophotometer

ELISA for rotavirus antibody The procedure described by Yolken et al (1) allowed reaction of a *guinea pig* anti rotavirus serum with a rotavirus positive stool filtrate the antigen was obtained by experimental infection of a gnotobiotic calf with human rotavirus This technique was modified as follows a ?% suspension of pooled stools from Costa Rican children positive for rotavirus by both electron microscopy and ELISA was added to each well of microtitre plates precoated with goat anti human rotavirus *antibody* ( ) Serial four fold dilutions of colostra were added in duplicate wells and the ELISA was continued as described above

Controls of ELISA All colostra were assayed for the presence of cholera toxin and rotavirus antigen The anti SC globulin was tested for the presence of antibodies

Acta Pædiatr Scand 68 161–164 1979

S IgA CHOLERA TOXIN AND ROTAVIRUS ANTIBODY IN HUMAN COLOSTRUM

ALBERTO SIMHON [1,2] ROBERT H YOLKEN and LEONARDO MATA [2]

From the Instituto de Investigaciones en Salud (INISA) Universidad de Costa Rica the [2]Ministerio de Salud San Jose Costa Rica and the [3]Laboratory of Infectious Diseases National Institute of Allergy and Infectious Diseases Bethesda Maryland USA

ABSTRACT Simhon A Yolken R H and Mata L (Instituto de Investigaciones en Salud (INISA) University of Costa Rica San Jose Costa Rica) S-IgA cholera toxin and rotavirus antibody in human colostrum Acta Paediatr Scand 68 161 1979.—S-IgA antibodies against cholera toxin and rotavirus were assayed in 43 colostral samples by means of an enzyme-linked immunosorbent assay (ELISA) All specimens contained specific S-IgA antibodies against both antigens Significant antibody titres to the antigens were demonstrated in almost all colostral samples

KEY WORDS Anti-cholera toxin anti rotavirus colostrum

The aetiology of non specific infantile diarrhoea has been further elucidated by recognition of the pathogenic role of enterotoxigenic *Escherichia coli* (5 12) and rotavirus (1 4) The risk of exposure of infants to these agents particularly in developing countries has stimulated study of the protective effect of breast feeding against their infection and associated diarrhoea

S IgA antibodies to enterotoxins of *Enterobacteriaceae* and *Vibrio cholerae* have been described in human milk by neutralisation assays and the enzyme linked immunosorbent assay (ELISA) (7 15) Also colostra of women from Costa Rica Guatemala and the United States were found to contain considerable amounts of anti rotavirus antibody (21) This report describes a semi quantitative assay of anti-cholera toxin S IgA antibody by ELISA as an alternative to neutralisation techniques Furthermore additional evidence is presented to confirm that colostra from Costa Rican women contain significant levels of anti rotavirus antibody

MATERIALS AND METHODS

Specimens Colostrum from 43 women aged 16 to 30 years from San José Costa Rica were obtained one to 72 hours post partum Mothers were of a medium to-low socio-economic status (approximate per capita yearly income US $70–150) All were at least minimally literate S IgA was assayed by the ELISA (7 17) in a modification developed by Yolken et al for rotavirus antigen (20) for labile toxin of *E coli* (19) and for rotavirus antibody assay (21)

ELISA for cholera toxin antibody 25 ng of cholera toxin (Schwarz Mann Inc Orangeburg N Y USA) were added to each well of polyvinyl microtitre plates (Cooke Engineering Co Alexandria Virginia USA) precoated with burro anti-cholera toxin antibody Six four fold dilutions of each colostrum sample were added in duplicate wells A conjugate of rabbit anti secretory component (SC) (Dakopatts AS Copenhagen Denmark) and alkaline phosphatase (Sigma Chemical Co St Louis Missouri USA) was then added to each well Addition of p-nitrophenyl phosphate substrate produced a yellow colour the reaction was stopped with 3N NaOH and the colour was read at 400 nm in a Beckman DU 2 Spectrophotometer

ELISA for rotavirus antibody The procedure described by Yolken et al (21) allowed reaction of a guinea pig anti rotavirus serum with a rotavirus positive stool filtrate the antigen was obtained by experimental infection of a gnotobiotic calf with human rotavirus This technique was modified as follows a 2% suspension of pooled stools from Costa Rican children positive for rotavirus by both electron microscopy and ELISA was added to each well of microtitre plates precoated with goat anti human rotavirus antibody (?) Serial four fold dilutions of colostra were added in duplicate wells and the ELISA was continued as described above

Controls of ELISA All colostra were assayed for the presence of cholera toxin and rotavirus antigen The anti SC globulin was tested for the presence of antibodies

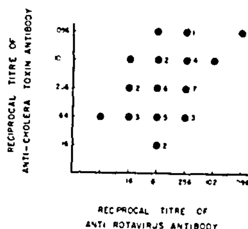


Fig. 1. Scatter diagram of reciprocal anti-cholera toxin and anti rotavirus antibody titres. 43 colostra from Costa Rican women 1978. Numeral indicates number of colostra.

to cholera toxin and rotavirus by means of blocking tests (20). In order to remove spurious antibodies to the various reagents in the tests (i.e. immune sera, colostra) those reagents were treated as described by Yolken et al. (22). Addition of colostrum was omitted in negative controls.

ELISA end point titres. To determine the titre of specific S IgA positive/negative (P/N) values were determined for each colostrum by dividing the spectrophotometric absorbance of the diluted sample by that of the negative control. A P/N value of 2.0 or greater was considered confirmatory of the presence of S IgA. Reciprocal antibody titres were defined as the highest dilution with a P/N value of 2.0 or greater.

RESULTS

None of the colostral samples contained either cholera toxin or rotavirus antigen. The anti SC globulin failed to block otherwise positive reactions for cholera toxin and rotavirus antigen.

All colostral samples were shown to contain S IgA antibodies to both cholera toxin and rotavirus antigen (Table I). The geometric mean titres of anti cholera toxin and anti rotavirus antibodies were 264.4 and 114.3 respectively. A scatter diagram of reciprocal titres for each colostrum is presented in Fig. 1. A positive correlating trend was observed.

DISCUSSION

The present findings reveal a high frequency of colostral specimens with significant titres of specific S IgA antibodies against choler[a] toxin. Since there has been no evidence [of] cholera in Costa Rica in this century it ca[n] be advanced that the labile toxin of *E. co[li]* (or of another *Enterobacteriaceae*) has bee[n] the immunogen eliciting anti cholera toxin antibody. The fact strengthens the resemblance [or] identity of these toxins (6, 13). Labile tox[in] (LT) of *E. coli* was demonstrated by passi[ve] immune hemolysis (3) in 9% of hospitalize[d] diarrhoeic and 6% of non diarrhoeic Cost[a] Rican children over a two year prospecti[ve] observation (11). Thus a high exposure to L[T] is observed in Costa Rica which presumabl[y] is reflected in high antibody titres. Enterotoxin neutralising activity was demonstrate[d] in a large number of specimens of Guatemala[n] colostrum and Pakistani mature milk b[y] Stoliar et al. (15) and Holmgren et al. (7) respectively. Inasmuch neutralisation titre[s] were not determined for the Costa Rica[n] colostrum no comparison can be made wit[h] such findings.

The ELISA geometric mean titre of choler[a] toxin S IgA in Pakistani milk was significantl[y] lower than that of Costa Rican colostrum presumably reflecting differences in concentration between milk and colostrum. Th[e] possibility that differences resulted from varying experimental conditions should not b[e] overlooked, e.g. crude or partially purifie[d]

Table I. *S IgA antibody to cholera toxin and rotavirus*

Reciprocal antibody titre	S IgA anti cholera toxin	S IgA anti rotavirus
4	0	1 (2)[b]
16	2 (5)[b]	6 (14)
64	12 (28)	16 (37)
256	15 (35)	15 (35)
1 024	11 (26)	4 (9)
4 096	3 (7)	1 (2)
Total	43	43

Highest dilution with a P/N value of 2 or greater (determined by dividing the spectrophotometric absorbance of the sample by that of the negative control).
[b] Number of colostra (percentage).

E coli heat labile enterotoxin was unavailable in our laboratory so commercially purified cholera toxin was utilized in this study anti body end point titres may have been defined differently in other studies also colostra from women of different socio economic condition may yield different results The present findings and those of Yolken et al (21) reveal significant amounts of anti rotavirus antibody in the majority of colostral specimens regardless of geographical location Furthermore they showed that rotavirus antibody persisted to detectable levels throughout 24 months of lactation Studies are under way to measure activity in terms of units of neutralisation per mg of S IgA

The correlation trend between titres of anti cholera toxin and anti rotavirus antibodies suggests a similar exposure or response in individual mothers to both antigens

In a model system of human rotavirus infection in lambs (14) passive immunity was studied by oral administration of human anti rotavirus IgG clinical manifestations were prevented presumably by neutralisation of either the initial inoculum or virus subsequently released by cells or both Nosocomial outbreaks of rotavirus have been controlled by oral administration of colostrum (16) Similarly exclusively breast fed Indian neonates and young infants of a Guatemalan Mayan village exhibited a lower incidence of gastrointestinal disorders despite frequent exposure to entero pathogens (9 10)

Regarding anti cholera factors protection against experimental cholera diarrhoea was shown to result from the synergistic action of anti bacterial and anti toxin antibodies (8) More research is needed to further asess the relative protective role of anti bacterial and anti toxin antibodies Colostral IgG and IgM antibodies already demonstrated in colostra from Central American women (18) also may provide added protection against enterotoxigenic and rotavirus infections

Evidently the study of immune principles active against newly recognised agents and other virulence factors is of public health significance especially in transitional societies now experimenting a decline in breast feeding Moreover the adequacy of passive immunity against toxigenic bacteria and rotaviruses must be evaluated in mature milk and in the various phases of lactation

ACKNOWLEDGEMENTS

This work was supported in part by the Vice Presidency of Research of the University of Costa Rica and the National Council for Scientific Research and Technology (CONICIT) Costa Rica The technical assistance of Patricia Munoz and Roberto Padilla is acknowledged

REFERENCES

1 Bishop R F Davidson G P Holmes I H & Ruck B J Virus particles in epithelial cells of duodenal mucosa from children with acute non bacterial gastroenteritis *Lancet II* 1281 1973

Engvall E & Perlmann P Enzyme linked immunosorbent assay (ELISA) III Quantitation of specific antibodies by enzyme labeled anti immunoglobulin in antigen coated tubes *J Immunol 109* 129 1972

3 Evans D J & Evans D G Direct serological assay for the heat labile enterotoxin of *Escherichia coli* using passive immune hemolysis *Infect Immun 16* 604 1977

4 Flewett T H Bryden A S & Davies H A Virus particles in gastroenteritis *Lancet II* 1497 1973

5 Gorbach S L Banwell J G Chatterjee B D Jacobs B & Sack R B Acute undifferentiated human diarrhea in the tropics I Alterations in intestinal microflora *J Clin Invest 50* 881 1971

6 Holmgren J Soderlind O & Wadstrom T Cross reactivity between heat labile enterotoxins of *Vib io chole ae* and *Escherichia coli* in neutralization tests in rabbit ileum and skin *Acta Path Microbiol Scand* Sect B *81* 757 1973

7 Holmgren J Hanson L A Carlsson B Lindblad B S & Rahimtoola J Neutralizing antibodies against *Escherichia col* and *Vibrio chole ae* enterotoxins in human milk from a developing country *Scand J Immunol 5* 867 1976

8 Holmgren J & Svennerholm A Mechanisms of disease and immunity in cholera a review *J Infect Dis 136* (Suppl) S105 1977

9 Mata L J & Urrutia J J Intestinal colonization of breast fed children in a rural area of low socioeconomic level *Ann N Y Acad Sci 176* 93 1971

10 Mata L J Kronmal R A Garcia B Butler W Urrutia J J & Murillo S Breast feeding weaning and the diarrhoeal syndrome in a Guatemalan Indian village *In* Ciba Foundation Symposium 4 (new series) *Acute diarrhoea in childhood* Elsevier Excerpta North Holland Amsterdam 1976 p 311

11 Reyes I Ramírez J A Evans D J & Mata L Escherichia coli enterotoxigénica en ninos hospitalizados de Costa Rica Rev Med Hosp Nac Ninos (Costa Rica) in press
12 Sack R B Gorbach S L Banwell J G Jacobs B Chatterjee B D & Mitra R C Enterotoxigenic *Escherichia coli* isolated from patients with severe cholera like disease *J Infect Dis 123* 378 1971
13 Smith N W & Sack R B Immunologic cross reactions of enterotoxins from *Escherichia coli* and *Vibrio cholerae J Infect Dis 127* 164 1973
14 Snodgrass D R Madeley C R Wells P W & Angus K W Human rotavirus in lambs infection and passive protection *Infect Immun 16* 268 1977
15 Stoliar O A Pelley R P Kaniecke Green E Klaus M H & Carpenter C C J Secretory IgA against enterotoxins in breast milk *Lancet I* 1258 1976
16 Totterdal B M Chrystie I L & Banatvala J E Rotavirus infection in a maternity unit *Arch Dis Child 51* 924 1976
17 Voller A Bidwell D & Bartlett A Microplate enzyme immunoassays for the immunodiagnosis of virus infections *In* N Rose & H Friedman (eds) *Manual of clinical immunology* American Society for Microbiology Washington D C 1976 p 506
18 Wyatt R B García B Cáceres A & Mata L J Immunoglobulins and antibodies in colostrum and milk of Guatemalan Mayan women *Arch Lat Am Nutr 22* 629 1972
19 Yolken R H Greenberg H B Merson M H Sack R B & Kapikian A Z Enzyme linked immunosorbent asssay for detection of *Escherichia coli* heat labile enterotoxin *J Clin Microbiol 6* 439 1977
20 Yolken R H Kim H W Clem T Wyatt R G Kalica A R Chanock R M & Kapikian A Z Enzyme linked immunosorbent assay (ELISA) for detection of human reovirus like agent of infantile gastroenteritis *Lancet II* 263 1977
21 Yolken R H Mata L García B Urrutia J J Wyatt R G Chanock R M & Kapikian A Z Secretory antibody directed against rotavirus in human milk measurement by means of an enzyme linked immunosorbent assay (ELISA) *J Pediatr* in press
22 Yolken R H Wyatt R G & Kapikian A Z ELISA for rotavirus *Lancet II* 819 1977

Submitted June 2 1978
Accepted Aug 31 1978

(L M) Instituto de Investigaciones en Salud (INISA)
Universidad de Costa Rica
San Jose
Costa Rica
America Central

Acta Pædiatr Scand 68 165–170 1979

DIETARY HABITS AND SERUM LIPIDS DURING FIRST 4 YEARS OF LIFE

A Study of 95 Danish Children

G E ANDERSEN C LIFSCHITZ and B FRIIS HANSEN

From the Neonatal Department Rigshospitalet Copenhagen Denmark

ABSTRACT Andersen G E. Lifschitz C and Friis Hansen B (Neonatal Department Rigshospitalet Copenhagen Denmark) Dietary habits and serum lipids during first 4 years of life A study of 95 Danish children Acta Paediatr Scand 68 165 1979.—Early infant feeding habits current dietary intake and serum lip us were invest gated in 31 infants age 6–10 months and 64 children age 3–4 years In the infants there was a correlation between serum lipid levels and the amount of saturated fat and the P/S ratio of the diet No such correlation was found in the 3–4 year old children Neither was there any correlation between the type and duration of early infant feeding and subsequent serum lipid levels In both the infants and the 3–4 year old children serum cholesterol concentration correlated with the serum cholesterol concentration in each of the parents

KEY WORDS Cholesterol triglyceride lipoprotein nutrition childhood

Hypercholesterolemia is a well documented risk factor in the development of coronary heart disease (1–3) In population studies serum cholesterol concentration has been found to correlate with the proportion of dietary calories derived from fat (4–9) particularly saturated fats (10) However studies which have tried to relate individual nutrient intake to serum cholesterol levels have failed to demonstrate any such correlation (11–15) The pioneer work of Brown & Goldstein (16) has shown that a substrate concentration of about 0 062 mmol/l of LDL C is sufficient to satisfy extrahepatic parenchymal cellular cholesterol requirements for membrane and hormone synthesis via high affinity LDL receptors The ratio of lymph to serum concentrations of LDL C has been calculated to be in the order of 1 10 (17) Therefore a lymph LDL C concentration of 0 062 mmol/l corresponds to a serum LDL C level of about 0 62 mmol/l which is close to the median level of cord serum LDL C found in normal newborns (18) and in eight mammalian species which are not naturally susceptible to atherosclerosis (19) In Western adults however the mean serum LDL C concentration is about 5 times higher i e 3 23 mmol/l (20) The question so far unresolved is how and when this five fold increase from birth to adult life takes place In order to contribute to the elucidation of this problem we here present data from a study of 95 normal children 64 of whom have been followed from birth to ages 3–4

MATERIAL

31 infants (13 girls and 18 boys) aged 6–10 months were randomly selected from our screening program for hyperlipoproteinemia (21) and 69 children (31 girls and 38 boys) aged 3–4 years were taken from a sample of 303 normal full term newborns who have earlier been described in detail (22) None of the 100 children here investigated have familial hypercholesterolemia (FH) The study and its purpose was explained to the parents and their consent obtained In cases of intercurrent disease the investigation of the child was postponed for a minimum of 2 weeks after the disappearance of symptoms to ensure that any possible drop in appetite and weight had returned to normal The daily nutritional intake of each child was evaluated by

---

Abbreviations T-C=total cholesterol VLDL-C=very low density lipoprotein cholesterol LDL C=low density lipoprotein cholesterol HDL-C=high density lipoprotein cholesterol TG=triglyceride P/S=ratio between the amount of polyunsaturated and saturated fat in the diet

Table 1 *Distribution of data in 6–10 month old infants (n=31)*

Percentile values	10	50	90
Age (months)	6 2	8 0	10 0
Weight (kg)	7 1	8 7	10 0
Weeks of breast feeding	0	6	18
Weeks of formula feeding	0	8	17
Weeks of diluted cow s milk feeding	0	4	8
Current daily dietary intake			
Kcals	704	918	1 265
Protein (g)	29 0	43 0	63 0
Carbohydrates (g)	84 0	111 0	226 0
Total fat (g)	15 2	30 0	53 6
Saturated fat (g) (S)	6 2	16 2	25 0
Polyunsaturated fat (g) (P)	1 3	3 0	5 8
P/S	0 09	0 20	0 40
Cholesterol (mg)	82	170	267
Current serum lipids (mmol/l)			
T C	3 36	4 22	5 52
VLDL C	0 06	0 20	0 47
LDL C	1 72	2 45	3 49
HDL C	1 14	1 67	2 19
TG	0 57	0 87	1 40

a one week dietary protocol written by the mother in combination with a final 24 hour dietary recall interview made by a dietitian The amounts of daily nutrients were then calculated according to a coded list of Danish food items (23) Furthermore the mother and the dietitian examined the child s health chart in which details of the type and duration of early infant feeding had been recorded by the visiting health nurse in collaboration with the mother On the morning of the interview a sample of venous blood was drawn from each child after an 8 hour fast (infants) or a 10–12 hour fast (3–4 year old children) In the parents a venous blood sample had been drawn at an earlier date after a 12 hour fast The serum was separated and stored at 4 C and the analyses begun within 24 hours In 2 children no venous blood sample could be taken and in 3 children information on dietary intake was incomplete thus resulting in a final total of 95 children

METHODS

Serum T–C was measured manually in duplicate using the enzymatic method described by Roschlau et al (24) Four Preciset cholesterol standards were included in over 100 separate runs The coefficient of variation was 4 4% (for the 0 32 mmol/l cholesterol Preciset) 1 7% (for the 2 59 mmol/l cholesterol Preciset) 1 9% (for the 3 88 mmol/l cholesterol Preciset) and 2 1% (for the 10 35 mmol/l cholesterol Preciset) Serum TG was measured manually in duplicate using the enzymatic method described by Eggstein & Kreutz (25) Two Liponorm triglyceride standards were included in over 100 separate runs The coefficient of variation was 3 6% (for the 0 42 mmol/l triglyceride Liponorm) and 2 0% (for the 2 50 mmol/l triglyceride Liponorm) VLDL LDL C was measured manually after $CaCl_2$ heparin precipitation in ... cate as described by Andersen & Gry Nielsen ... VLDL C was measured manually in duplicate after centrifugation in a 40 3 rotor Beckman type L ultr... trifuge at 10°C at 40000 r p m for 20 hours Tube ... was used HDL values were calculated as the diffe... between (T C)–(VLDL LDL C)

Statistics

The distribution of data in the two groups of childre... determined by calculating the percentile values The ... tionship between the child s current serum lipid level ... his past serum cholesterol serum cholesterol in ... parents early infant feeding and daily nutrient intake ... evaluated by calculating the Spearman rank correl... coefficients and conducting the appropriate T tes... described by Siegel (27) The Mann Whitney test was ... for comparing serum lipid values in the 6–10 months ... infants and the 3–4 year old children as describe... Siegel (27)

RESULTS

6–10 months old infants

In Table 1 the distributions of age weight t... and duration of early infant feeding curr... dietary intake and serum lipids are given ... the 31 infants Only 2 infants were pur... breast fed for >20 weeks (28 and 32 wee... resp) They were still being breast fed at t... time their serum lipids were determined Thr...

Table 2 6–10 month old infants Correlations between current serum T C and serum T C at birth serum T C of the parents early infant feeding and current dietary intake (n=31)

	r	*p*
Child s own cord serum T-C	−0 080	N S
Child s own cord serum LDL C	0 ??1	N S
Mother s age-corrected T C	0 335	<0 05
Father s age corrected T C	0 311	<0 05
Weeks of breast feeding	0 006	N S
Weeks of formula feeding	0 0?9	N S
Weeks of diluted cow s milk feeding	−0 049	N S
Cu rent daily dietary intake		
Kcal	−0 141	N S
Protein	−0 ?86	N S
Carbohydrates	−0 54	N S
Total fat	0 85	N S
Saturated fat	0 372	<0 05
Polyunsaturated fat	−0 ? 0	N S
P/S	−0 630	<0 0001
Cholesterol	0 ?07	N S

infants were purely formula fed for 20–24 weeks The remaining 26 infants were first breast fed and then received formula and/or diluted cow s milk In Table 2 the relationships between the current serum T C of the child and his cord serum T C and LDL C the age adjusted serum T C of his parents the duration of breast formula and diluted cow s milk feeding and the amount of nutrients in his current diet are presented A correlation is seen to exist between the current serum T C of the child and that of his parents Furthermore the current serum T C level is seen to correlate directly with the amount of saturated fat and inversely with the *P/S ratio of the diet* Exactly the same pattern emerged when the infant s current serum LDL C was compared to the factors given in Table 2

As for serum TG an inverse correlation was found with the amount of fat in the diet ($r=-0\ 436$ $p<0\ 01$) and the amount of dietary cholesterol ($r=-0\ 479$ $p<0\ 005$) but not with the amount of dietary carbohydrate ($r=0\ 104$ $p>0\ 05$)

No difference was found in the distribution of any of the serum lipids in the 13 girls and 18 boys

3–4 year old children

In Table 3 the distribution of age weight type and duration of early infant feeding current dietary intake and serum lipids are given for

Table 3 *Distribution of data in 3–4 year old children (n=64)*

Percentile values	5	10	50	90	95
Age (months)	36	37	47 5	50	50
Weight (kg)	14 5	15 1	17 5	0 7	21 4
Weeks of breast feeding	0	0	8	16	20
Weeks of *formula feeding*	0	0	8	?0	?0
Weeks of diluted cow s milk feeding	0	0	0	1?	14
Cu ent da ly d etary intake					
Kcal	981	1 063	1 500	000	. 181
Protein (g)	38	46	65	8?	90
Carbohydrates (g)	96	103	157	236	?76
Total fat (g)	36 .	40 0	66 0	97 4	1.5 2
Saturated fat (g)	14 6	17 7	9 5	45 0	53 0
Polyunsaturated fat (g)	3 0	4 9	8 9	15 1	?1 8
P/S	0 15	0 17	0 3?	0 58	0 68
Cholesterol (mg)	134	1 0	90	449	50.
Current serum lipids (mm ol/l)					
T-C	3 40	3 69	4 7?	5 75	6 07
VLDL-C	0 04	0 05	0 1.	0 ?8	0 34
LDL-C	1 51	1 9?	47	3 43	3 8?
HDL-C	1 6	1 49	? 05	. 5?	2 8?
TG	0 49	0 51	0 65	0 89	1 12

Table 4 3–4 year old children Correlations between current serum T C and serum T C at birth 7–9 and 14–19 months serum T C of the parents early infant feeding and current dietary intake (n=64)

	r	p
Child s cord serum T C	−0 144	N S
Child s serum T C at 7–9 months	0 450	<0 0005
Child s serum T C at 14–19 months	0 517	<0 00005
Mother s age corrected T C	0 380	<0 001
Father s age corrected T C	0 350	<0 005
Weeks of breast feeding	−0 091	N S
Weeks of formula feeding	0 057	N S
Weeks of diluted cow s milk feeding	0 038	N S
Current daily dietary intake		
Kcals	0 021	N S
Protein	0 004	N S
Carbohydrates	0 076	N S
Total fat	−0 066	N S
Saturated fat	0 057	N S
Polyunsaturated fat	−0 005	N S
P/S	−0 150	N S
Cholesterol	0 067	N S

the 64 children In Table 4 the relationships between the current serum T C of the child and his past serum T C the age corrected serum T C in each of his parents the duration of breast formula and diluted cow s milk feeding and the amount of daily nutrients of his diet are presented Correlations are seen to exist between the current serum T C level of the child and his serum T C at age 7–9 and 14–19 months and the serum T C of each of his parents However no correlations were found between either his current serum T C and the duration of breast milk formula and diluted cow s milk feeding or between his current serum T C and the individual nutrients of his diet Exactly the same pattern emerged when the child s current serum LDL C was compared to the factors given in Table 4

As for serum TG a correlation was found with the serum TG at age 7–9 months ($r=0\ 282$ $p<0\ 05$) and at age 14–19 months ($r=0\ 291$ $p<0\ 05$) No correlation was found between serum TG and the individual nutrients of the diet No difference was found in the distribution of any of the serum lipids in the 28 girls and 36 boys

A comparison of the serum lipids in the two groups of children showed that in the 3–4 year old children serum T C was higher ($p<0\ 05$) TG lower ($p<0\ 001$) VLDL-C lower ($p<0\ 005$) and HDL C higher ($p<0\ 001$) than in the 6–10 months old infants No difference was found between serum LDL-C levels in the two groups of children ($p=0\ 836$)

DISCUSSION

The median value for serum T C (4 22 mmol/l) in our 6–10 months old infants is of the same order of magnitude as the mean values for serum T-C (3 78–5 06 mmol/l) in infants on various diets found by Fomon & Bartels (28) Paupe et al (29) Woodruff et al (30) Darmady et al (31) and Fosbrooke & Wharton (32)

The median value for serum T C (4 7? mmol/l) in our 3–4 year old children is 16% higher than the mean value for serum T-C (3 98 mmol/l) in 3 year old children reported by Friedman & Goldberg (33) This discrepancy may partly be due to the use of different methods for measuring T C Our finding that there is no association between the type and duration of early infant feeding and subsequent serum T C be it after as short as 3 months or as long as 3 years confirms the results of Friedman & Goldberg (34) and Glueck et al (35) and is one further proof that the hypothesis of Reiser et al (36) is incorrect Their hypothesis derived from experiments with infant male rats and pigs was that the feeding of a high cholesterol diet to the human infant might cause subsequent low values of serum T C based on the speculation that an early high cholesterol diet might induce life long high enzyme activities in cholesterol catabolism

Our finding that in 6–10 months old infants there is a direct association between the amount of saturated fat and an inverse relationship between the P/S ratio of the diet and the level of serum T C is in agreement with what has been reported by Fomon & Bartels

28) Goalwin & Pomeranze (37) Paupe et al 29) Lowe et al (38) Glueck et al (35) Dar mady et al (31) and Friedman & Goldberg (34)

Our finding that beyond infancy there is no association between the amounts of daily nutrients and the concentration of serum T C confirms the results of Hard & Esselbaugh (39) and Hitchcock & Gracey (15) and Weidman et al (40) in older children

This report is the first which has shown that the increase in serum T C levels between in fancy (6–10 months) and age 3–4 years is caused by an increase in HDL C Serum LDL C was found to be the same in the two age groups

In conclusion we have found that about 3/5 of the 5 fold increase in serum LDL C from birth to adult life takes place in early infancy In infants the type and amount of fat in the diet seem to influence the level of serum T C and TG This influence however seems to be lost beyond infancy since in 3–4 year old children serum T C and TG do not correlate with die tary intake Both during infancy and later childhood there is a correlation between serum T C of the child and his parents This might be interpreted in the following way During in fancy serum T C seems to be under at least both genetic and dietary influence beyond infancy however the dietary influence sub sides whereas the genetic influence seems to persist

ACKNOWLEDGEMENT

This study was made possible by grants from the Danish Heart Foundation and the Dagmar Marshall Fund We thank the dietitians of Department P and G of the Rigs hospital for their invaluable help in making the dietary recalls and calculations of daily dietary intake

REFERENCES

1 Kannel W B Castelli W P Gordon T & M Namara M Serum cholesterol lipoproteins and the risk of coronary heart disease The Framingham study *Ann Int Med 74* 1 1971

Chapman J M & Massey F J The interrelationship of serum cholesterol hypertension body weight and risk of coronary disease *J Chron Dis 17* 933 1964

3 Rosenman R H Friedman M Strauss R Jenkins C D Zyzanski S J & Wurm M Coronary heart disease in the Western Collaborative Group Study *J Chron Dis 23* 173 1970

4 Keys A Anderson J T & Grande F Prediction of serum cholesterol response of man to changes in fats in the diet *Lancet II* 959 1957

5 Connor W E Stone D B & Hodges R E The interrelated effects of dietary cholesterol and fat upon human serum lipid levels *J Clin Invest 43* 1691 1964

6 National diet–heart study research group The national–diet–heart study final report *Circulation 37* (Suppl I) 1 1968

7 Keys A Coronary heart disease in seven countries *Circulation 41* (Suppl I) 1 1970

8 Kato H Tillotson J Nichaman M Z Rhoads G & Hamilton H Epidemiological studies of coronary heart disease and stroke in Japanese men living in Japan Hawaii and California *Am J Epidemiol 97* 372 1973

9 Hankin J Reed D Labarthe D Nichaman M & Stallones R Dietary and disease patterns among Micronesians *Am J Clin Nutr 23* 346 1970

10 Atherosclerosis study group Primary prevention of the atherosclerotic diseases Report of intersociety commission for heart disease resources *Circulation 42* (Suppl A) 1 1970

11 Kannel W & Gordon T *The Framingham study* An epidemiological investigation of cardiovascular diseases Section 24 The Framingham diet study Diet and the regulation of serum cholesterol U S Government Printing Office 1970

12 Kahn H Medalie J Neufeld H Riss E Balogh M & Groen J Serum cholesterol Its distribution and association with dietary and other variables in a survey of 10000 men *Israel J Med Sci 5* 1117 1969

13 Stulb S McDonough T Greenberg B & Hames C G The relationship of nutrient intake and exercise to serum cholesterol levels in white males in Evans County Georgia *Am J Clin Nutr 16* 238 1965

14 Nichols A B Ravenscroft C Lamphiear D E & Ostrander L D Daily nutritional intake and serum lipid levels The Tecumseh study *Am J Clin Nutr 29* 1384 1976

15 Hitchcock N E & Gracey M Diet and serum cholesterol An Australian family study *Arch Dis Child 52* 790 1977

16 Goldstein J L & Brown M Atherosclerosis The low-density lipoprotein receptor hypothesis *Metabolism 26* 1257 1977

17 Reichl D Simons L A & Myant N B The lipids and lipoproteins of human peripheral lymph with observations on the transport of cholesterol from plasma and tissues into lymph *Clin Sci Mol Med 45* 313 1973

18 Andersen G E & Friis Hansen B Cord serum lipid and lipoprotein-cholesterol values in normal and betamethasone treated newborns of varying gestational age *Acta Paediat Scand 66* 355 1977

Table 4 *3–4 year old children Correlations between current serum T C and serum T C at birth 7–9 and 14–19 months serum T C of the parents early infant feeding and current dietary intake (n=64)*

	r	p
Child s cord serum T C	−0 144	N S
Child s serum T C at 7–9 months	0 450	<0 0005
Child s serum T C at 14–19 months	0 517	<0 00005
Mother s age corrected T C	0 380	<0 001
Father s age corrected T C	0 350	<0 005
Weeks of breast feeding	−0 091	N S
Weeks of formula feeding	0 057	N S
Weeks of diluted cow s milk feeding	0 038	N S
Current daily dietary intake		
Kcals	0 021	N S
Protein	0 004	N S
Carbohydrates	0 076	N S
Total fat	−0 066	N S
Saturated fat	0 057	N S
Polyunsaturated fat	−0 005	N S
P/S	−0 150	N S
Cholesterol	0 067	N S

the 64 children In Table 4 the relationships between the current serum T C of the child and his past serum T C the age corrected serum T C in each of his parents the duration of breast formula and diluted cow s milk feeding and the amount of daily nutrients of his diet are presented Correlations are seen to exist between the current serum T C level of the child and his serum T C at age 7–9 and 14–19 months and the serum T C of each of his parents However no correlations were found between either his current serum T C and the duration of breast milk formula and diluted cow s milk feeding or between his current serum T C and the individual nutrients of his diet Exactly the same pattern emerged when the child s current serum LDL C was compared to the factors given in Table 4

As for serum TG a correlation was found with the serum TG at age 7–9 months ($r=0\ 282$ $p<0\ 05$) and at age 14–19 months ($r=0\ 291$ $p<0\ 05$) No correlation was found between serum TG and the individual nutrients of the diet No difference was found in the distribution of any of the serum lipids in the 28 gr and 36 boys

A comparison of the serum lipids in the tw groups of children showed that in the 3 year old children serum T C was high ($p<0\ 05$) TG lower ($p<0\ 001$) VLDL lower ($p<0\ 005$) and HDL C higher ($p<0\ 0$ than in the 6–10 months old infants No d ference was found between serum LDL levels in the two groups of children ($p=0\ 8$

DISCUSSION

The median value for serum T C (4 22 mmo in our 6–10 months old infants is of the sa order of magnitude as the mean values for rum T C (3 78–5 06 mmol/l) in infants on v ious diets found by Fomon & Bartels (Paupe et al (29) Woodruff et al (30) Dar dy et al (31) and Fosbrooke & Wharton (3

The median value for serum T C (mmol/l) in our 3–4 year old children is 1 higher than the mean value for serum (3 98 mmol/l) in 3 year old children reporte Friedman & Goldberg (33) This discrepa may partly be due to the use of different m ods for measuring T C Our finding that t is no association between the type and d tion of early infant feeding and subsequent rum T C be it after as short as 3 months c long as 3 years confirms the results of Fr man & Goldberg (34) and Glueck et al (35) is one further proof that the hypothesis of ser et al (36) is incorrect Their hypoth derived from experiments with infant male and pigs was that the feeding of a high cho terol diet to the human infant might ca subsequent low values of serum T C base the speculation that an early high cholest diet might induce life long high enzyme ac ties in cholesterol catabolism

Our finding that in 6–10 months old infa there is a direct association between amount of saturated fat and an inverse r tionship between the P/S ratio of the diet the level of serum T C is in agreement w what has been reported by Fomon & Bar

H_2 BREATH TESTS DURING DIARRHEA

N W SOLOMONS R GARCÍA R SCHNEIDER F E VITERI and
V ARGUETA von KAENEL

From the Division of Human Nutrition and Biology Institute of Nutrition of Central America and Panama and the Department of Paediatrics San Juan de Dios General Hospital Guatemala City Guatemala

ABSTRACT Solomons N W Garcia R Schneider R Viteri F and Argueta von Kaenel V (Division of Human Nutrition and Biology Institute of Nutrition of Central America and Panama and Department of Pediatrics San Juan de Dios General Hospital Guatemala City Guatemala) H_2 breath test during diarrhea Acta Paediatr Scand 68 171 1979.—The peak rise in breath hydrogen and the volume of excess pulmonary excretion of hydrogen in response to a 10 g dose of the non absorbable disaccharide lactulose was significantly lower in children with active gastroenteritis and diarrhea than in non diarrheal controls Thus despite the fact that the H_2 breath test is a convenient non invasive technology for use in children it cannot be recommended for measuring carbohydrate malabsorption in individuals with active on-going episodes of diarrhea

KEY WORDS Hydrogen breath test carbohydrate malabsorption infantile diarrhea lactulose

When ingested carbohydrate is exposed to certain colonic bacteria fermentation results in the intraintestinal production of hydrogen (H_2) some of this H_2 is excreted by the lungs Since a linear and relatively constant relationship between the amount of non absorbed carbohydrate and the rate of H_2 excretion has been demonstrated (1) clinical carbohydrate malabsorption tests based on breath analysis have been developed The innocuous non invasive nature of the collection procedure as compared with other techniques for quantifying carbohydrate malabsorption has encouraged application of the H_2 methodology in young children (2 4 6)

We have been interested in determining the amount of nutrient loss which can occur as the result of acute infantile gastroenteritis and felt that the H_2 breath test would be useful in the evaluation of this problem However our initial experience with administering a 15 g oral glucose dose to infants with intractable diarrhea and clinical monosaccharide intolerance or with giving 1 75 g of lactose per kg to children with acute enteritis failed to show the expected rise in breath H_2 where large amounts of fecal reducing substances could be detected in the stools (Solomons Garcia and Viteri unpublished) Moreover in some institutionalized children followed prospectively a drastic reduction in the usual rate of pulmonary H elimination on a normal diet accompanied episodes of diarrhea (Schneider unpublished) These curious results led us to examine the question of whether or not the presence of diarrhea itself was affecting the pulmonary excretion of H_2

PATIENTS AND METHODS

Five pre school aged children without diarrhea in the Clinical Research Center of the Division of Human Nutrition and Biology Institute of Nutrition of Central America and Panama served as controls Ten children aged 6 months to 3 years selected at random from among patients admitted to the Rehydration Unit of the San Juan de Dios General Hospital with acute gastroenteritis had ongoing active diarrhea Only 3 of the latter subjects had received antibiotics prior to study All subjects received 10 g of the non absorbable disaccharide lactulose Samples of expired air were collected before and at 30-min intervals following the administration of the lactulose dose for a total of 4 hours and the breath H_2 concentration was measured by gas chromatography as previously described (5) The pulmonary excretion of

19 Mills G L & Taylor C L The distribution and composition of serum lipoproteins in eighteen animals *Comp Biochem Physiol 40B* 489 1971
20 Fredrickson D S & Levy R I *In* J B Stanbury J B Wyngaarden & D S Fredrickson (eds) *The metabolic basis of inherited disease* McGraw Hill New York 1972 p 547
21 Andersen G E Lous P & Friis Hansen B screening for hyperlipoproteinemia in 10000 Danish newborns *Acta Paediatr Scand 68* 1979 In press
22 Andersen G E & Friis Hansen B Neonatal diagnosis of familial type II hyperlipoproteinemia *Pediatrics 57* 214 1976
23 Helms P *Fødevaretabeller* Akademisk Forlag Copenhagen 1975
24 Röschlau P Bernt E & Gruber W Enzymatische Bestimmung des Gesamt Cholesterins im Serum *Z Klin Chem Klin Biochem 12* 4 1974
25 Eggstein M & Kreutz F H Eine neue Bestimmung der Neutralfette im Blutserum und Gewebe *Klin Wochenschr 44* 262 1966
26 Andersen G E & Gry Nielsen H Neonatal screening for hyperlipoproteinemia Methods for direct estimation of cord serum VLDL LDL *Clin Chim Acta 66* 29 1976
27 Siegel S *In* S Siegel (ed) *Nonparametric statistics for the behavioral sciences* McGraw Hill New York 1956 p 203 and 116
28 Fomon S J & Bartels D J Concentrations of cholesterol in serum of infants in relation to diet *Am J Dis Child 99* 27 1960
29 Paupe J Colin J & Halvorson Politis E La cholestérolemie du nouveau né et du nourisson normaux jusqu à 2 ans *Arch Fr Pediatr 20* 513 1963
30 Woodruff C W Bailey M C Davis J T Rogers N & Coniglio J G Serum lipids in breast fed infants and in infants fed evaporated milk *Am J Clin Nutr 14* 83 1964
31 Darmady J M Fosbrooke A S & Lloyd J K Prospective study of serum cholesterol levels during first year of life *Br Med J II* 685 1972
32 Fosbrooke A S & Wharton B A Added lacto[se] and added sucrose cow s milk formulae in n[...] of low birthweight babies *Arch Dis Child 50* 4[...] 1975
33 Friedman G & Goldberg S J An evaluation of [the] safety of a low saturated fat low-cholesterol diet [be]ginning in infancy *Pediatr 58* 655 1976
34 Friedman G & Goldberg S J Concurrent and [sub]sequent serum cholesterols of breast and formula infants *Am J Clin Nutr Invest 28* 42 1975
35 Glueck C J Tsang R Balistreri W & Fallat [...] Plasma and dietary cholesterol in infancy Effect[s of] early low or moderate dietary cholesterol intak[e on] subsequent response to increased dietary cholest[erol] *Metabolism 21* 1181 1972
36 Reiser R & Sidelman Z Control of serum ch[oles]terol homeostasis by cholesterol in the milk of suckling rat *J Nutr 102* 1009 1972
37 Goalwin A & Pomeranze J Serum chole[sterol] studies in infants *Arch Paediatr 79* 58 196[...]
38 Lowe C U Mosovich L L & Pessin V Ef[fect] of protein level and type of heat treatment of formulas on growth and maturation of infan[ts] *Pediatr 64* 666 1964
39 Hard M M & Esselbaugh N C Nutritional s[tatus] of adolescent children IV Cholesterol relation[s] *Am J Clin Nutr 8* 346 1960
40 Weidman W H Elveback L R Nelson R Hodgson P A & Ellefson R D Nutrient i[ntake] and serum cholesterol level in normal children [...] years of age *Pediatrics 61* 354 1978

Submitted May 17 1978
Accepted June 20 1978

(G E A) Neonatal Department GN 5024
Rigshospitalet
Blegdamsvej 9
DK 2100 Copenhagen
Denmark

Acta Pædiatr Scand 68 173–180 1979

RENAL FUNCTIONAL CHANGES IN ACUTE GLOMERULONEPHRITIS IN CHILDREN A ONE YEAR FOLLOW UP

A APERIA A BERGSTRAND O BROBERGER T LINNÉ and J WASSERMAN

From the Department of Paediatrics Karolinska Institutet St Goran s Children s Hospital the Department of Pathology Karolinska Institutet Huddinge Hospital and the Central Microbiological Laboratory Stockholm County Council Stockholm Sweden

ABSTRACT Aperia A Bergstrand A Broberger O Linne T and Wasserman J (Department of Paediatrics Karolinska Institutet St Goran s Children s Hospital Department of Pathology Karolinska Institutet Huddinge Hospital and the Central Microbiological Laboratory Stockholm County Council Stockholm Sweden) Renal functional changes in acute glomerulonephritis in children A one-year follow up Acta Paediatr Scand 68 173 1979.—Renal function was studied in three patients with post streptococcal four patients with IgA and one patient with non streptococcal proliferative glomerulonephritis (GN) at the onset of the disease and two six and 12 months later Renal biopsies were performed at the onset of the disease and 12 months later Standard clearance techniques were used for the functional studies The latter were performed during hydropenia and continuous isotonic saline infusion During hydropenia the GFR was uniformly depressed shortly after the onset of the disease but it normalized during the following two months The filtration fraction was depressed in poststreptococcal GN at the onset and it normalized with the GFR In IgA GN the filtration fraction remained within normal limits during the entire course of the illness The natriuretic response to isotonic saline volume expansion was low in all patients at the onset of the d sease but normalized in post-streptococcal and IgA GN during the one year follow up In spite of normalized renal function biopsy findings in IgA GN were unchanged 12 months later An episode of macroscopic hematuria in one patient with IgA GN at the six month investigation had no apparent effect on renal function

KEY WORDS Post-streptococcal glomerulonephritis IgA glomerulonephritis renal function Isotonic saline infusion one-year clinical course

Glomerulonephritis (GN) is the most common cause of chronic renal failure in children (14) and adults (6) The natural course of the different types of GN is incompletely known however and our ability to identify patients at risk of developing progressive renal damage is unsatisfactory The pathophysiology of the hypertension seen in acute and chronic GN also needs to be clarified Systematic prospective studies of patients with well-defined GN from the onset of the disease should help us to better understand the disease and its consequences and enable us to handle clinical problems more satisfactorily In order to see what information could be obtained by an intensive one year follow up study of acute GN eight children were serially examined from the onset of the disease and at two six and 12 months Data were obtained on the clinical course renal function and renal pathology

MATERIAL

The studies were performed during a two-year period (1973–75) on patients who were admitted to St Goran s Children s Hospital with the preliminary diagnosis of acute GN The patients were admitted to the study if they fulfilled the following criteria 1) no previous history of renal disease 2) no signs or symptoms of systemic disease such as anaphylactoid purpura or systemic lupus erythematosus 3) acute onset of longstanding macroscopic hematuria with proteinuria for at least one week and 4) negative urine culture and normal intravenous pyelogram The diagnosis of acute glomerular disease was further confirmed by the clinical course laboratory findings and the results of renal biopsy

Eight patients aged 4 5 to 9 5 years were studied Three

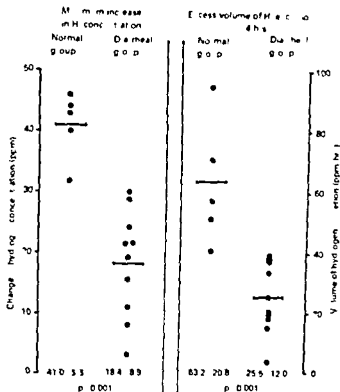


Fig 1 Scattergram illustrating the breath hydrogen response to ingestion of 10 g of lactulose in healthy children and in children with diarrhea Hydrogen excretion has been quantified both as the maximum increase in H_2 concentration in ppm and as the excess volume of H_2 in ppm hrs over 4 hours

H_2 was compared in two ways 1) as the maximum increase in H_2 concentration in ppm and 2) as the volume of H_2 eliminated during the 4 hour period in ppm hrs

RESULTS AND DISCUSSION

As shown in Fig 1 a significant reduction ($p<0\ 001$) in H excretion to the same 10 g dose of lactulose was seen among children with acute diarrhea as compared with normal control children The explanation is clear but several mechanisms could be involved First ly quantitative or qualitative changes in fecal flora during diarrhea could reduce the critical mass of appropriate bacteria necessary for fermentation of the carbohydrate substrate Alternatively changes in colonic motility during active diarrhea could change the partition of colonic H excretion between breath and flatus with a proportionately greater amount of intestinal H_2 being excreted by rectum We and others previously shown that oral antibiotics can interfere with the bacterial metabolism and H excretion from carbohydrates (3 5) but in only 3 of the 10 subjects was prior use of antibiotics a factor

The H breath test can be a valuable and reliable technology for the study of carbohydrate malabsorption Its non invasive nature makes it particularly suited to studies in young children The important conclusion of the present study is that standard H breath test criteria for carbohydrate malabsorption based on normal subjects cannot be reliably applied to individuals with active gastroenteritis This would seem to render the H breath analysis techniques inapplicable to the study of carbohydrate absorption in diarrheal disease

ACKNOWLEDGEMENTS

The authors would like to thank the nursing staff of the Clinical Research Center of INCAP and of the Pediatric Emergency Service of the San Juan de Dios General Hospital Dr Solomons was a recipient of the Josiah Macy Jr Foundation Faculty Fellowship and of the Future Leaders Award of the Nutrition Foundation

REFERENCES

1 Bond J H & Levitt M D Use of pulmonary hydrogen (H_2) measurements to quantitate carbohydrate absorption Study of partially gastrectomized patients *J Clin Invest 51* 1219 1972
2 Maffei H V L Metz G L & Jenkins D J A Hydrogen breath test Adaptation of 2 simple techniques to infants and children *Lancet I* 1110 1976
3 Murphy E L & Calloway D H The effect of antibiotic drugs on the volume and composition of intestinal gas from beans *Am J Dig Dis 17* 6 1972
4 Perman J A Barr R G & Watkins J B Sucrose malabsorption in children Non invasive diagnosis by interval breath hydrogen determination *J Pediatr* (in press)
5 Solomons N W Viteri F E & Hamilton L H Application of a simple gas chromatographic technique for measuring breath hydrogen *J Lab Clin Med 9*[illegible] 856 1977
6 Solomons N W Viteri F E & Rosenberg I H Development of an interval sampling hydrogen (H) breath test for carbohydrate (CHO) malabsorption in children Evidence for a circadian pattern of H_2 concentration *Pediatr Res 12* 816 1978

Submitted June 21 1978
Accepted Aug 28 1978

(N W S) Division of Human Nutrition and Biology
INCAP
Carretera Roosevelt Zona 11
Guatemala C A AP 11-88
INCAP publication I 1010

Table 1 *Glomerular filtration rate (ml/1 73 m BSA/min) in acute glomerulonephritis*
Values are mean ± S E M HP=hydropenia VE=volume expansion

	Onset		12 months later		Difference onset–12 months later	
	HP	VE	HP	VE	HP	VE
Poststreptococcal GN	73 9±5 4	92 3±18 2	99 8±1 4	100 9±6 ?	+ 6 9 p<0 01	+8 8 NS
IgA GN	74 9±6 6	107 3±13 ?	96 5±5 ?	103 4±3 9	+?1 6 p<0 01	−3 9 NS
Non streptococcal prolif GN	68 0	91 7	66 4	101 9	−1 6	+10 2

RESULTS

Renal biopsy

Biopsy material was available only from one patient with PSGN (T E) one month after the onset of the disease Slight mesangial hypercellularity and increased amounts of matrix was seen with segmental and focal distribution In some glomeruli the capillary tuft had increased lobulation IFL was positive only for C3 with mesangial localization

Three (P H K J O M) of the patients with IgA GN were biopsied one to three months after the onset of hematuria In all cases there was a slight to moderate increase in mesangial matrix without hypercellularity in most glomeruli After 12 months the lesions were unchanged in two of the patients (P H K J) and in the third (O M) an additional segmental sclerosis was found The fourth patient (A N) who was biopsied for the first time at 12 months had minimal changes IFL examination showed granular deposits of IgA IgG and C3 predominantly in the mesangium but also along capillary glomerular basement membranes In two cases IgM was present (P H K J) No change in the IFL pattern was noted between the first and second biopsies (P H K J)

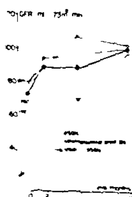


Fig 1a The GFR during HP in PS and non streptococcal proliferative GN (open circles) shortly after the onset of the disease and two six and 12 months later The individual values are connected by thin continuous lines in PS and by an interrupted line in non streptococcal proliferative GN The closed circles and connecting thick line represent the means in PS GN



Fig 1b The GFR during HP in IgA GN shortly after the onset of the disease and two six and 12 months later The means (closed circles) and individual values (open circles) are shown

patients (one boy two girls) were classified as post streptococcal GN four (three boys one girl) as IgA GN and one (boy) as non streptococcal proliferative GN The criteria used for the subclassification were high ASO titers and C3 depression in post streptococcal GN (PSGN) recurrent macroscopic hematuria and deposits of IgA predominantly in the glomerular mesangium in IgA GN and stationary ASO titers longstanding depression of C3 and renal biopsy findings in non streptococcal proliferative GN

Two (R F A H) of the three patients with *post streptococcal GN* had increased S urea N during the first days of the disease This was in one case (R F) associated with oliguria and edema but not hypertension All the patients showed initial good recovery Blood pressure blood volume bromide space recumbent renin and aldosterone excretion were essentially within normal limits during the entire follow up period Urinalysis was normal in all cases 12 months after the onset of the disease In connection with a non streptococcal upper respiratory tract infection one month after the onset of the disease one patient (R F) had an exacerbation with increased S urea N for one month

Two (K J A N) of the four patients classified as *IgA GN* had increased S urea N early in the course None of the patients had visible edema but one boy (A N) aged 4 5 years had a borderline elevation of the blood pressure (130/80) Renin and aldosterone values were slightly elevated in two (P H O M) of the patients at the onset Moderate proteinuria (max 2 4–4 5 g/l) was observed 1 5–2 5 months in three patients (P H K J A N) and was still present in one patient (O M) after 12 months Recurrences of macroscopic hematuria occurred in immediate connection with upper respiratory tract infections and was usually of only a few days duration In one patient (O M) a follow up examination at six months was carried out during an episode of macroscopic hematuria Microscopic hematuria was a constant finding in all patients during the entire observation period Serum urea N blood pressure blood volume bromide space renin and aldosterone were consistently within normal limits after the initial phase of the disease

The patient with *non streptococcal proliferative GN* had an upper respiratory tract infection one week before the onset of macroscopic hematuria ASO and anti DNase B titers were repeatedly normal C3 was depressed for about eight months Oliguria edema and hypertension were not seen in the early or later stages of the disease Serum urea N was elevated during the first two months and proteinuria (max 5 0 g/l) was present for 2 5 months Blood volume bromide space recumbent renin and aldosterone excretion were within normal limits throughout the year Towards the end of the follow up period urinalysis became normal

METHODS

The patients were studied in the acute phase and two six and 12 months thereafter The studies at the first admission were started as soon as possible but were not performed until the S urea N was normal or close to normal The same protocol was used at each time except for renal biopsies which in most cases were performed in the acute phase and one year later

The patients were placed on a normal diet with 100–150 mEq Na /1 73 m² body surface area (BSA) d for at least four days before the study was started Blood pressure was taken repeatedly over a 24 hour period with an arm cuff and mean values for systolic and diastolic pressures were calculated Blood volume was determined using a diluting technique with ^{131}I albumin The bromide space was measured with a nuclear activation technique (5) The fasting morning levels of renin (7) and the 24-hour excretion of aldosterone (9 10) were also determined

Renal biopsy

Percutaneous renal biopsies with a Tru Cut* needle were performed under general anesthesia No complications occurred The biopsy material was prepared for examination by light microscopy and immunofluorescence (IFL) using the methods described by Berg et al (3)

The following fluorescent conjugates were used routinely heterospecific anti human immunoglobulin (National Bacteriological Lab Stockholm) and anti human IgG IgM IgA C3 and fibrinogen (Hyland Lab Costa Mesa Calif)

Renal function studies

Standard clearance techniques in conjunction with bladder catheterization were used The studies were started after at least 17 hours of food and fluid deprivation Inulin or Inutest and PAH were first administered in a prime dose of 0 5 ml/kg body wt of 45 ml 10% Inulin Laevosan Gesellschaft + 5 ml 20% Sodium aminohippurate MSD or 0 3 ml/kg body wt of 17 ml 25% Inutest Laevosan Gesellschaft + 3 ml 20% Sodium aminohippurate MSD This was followed by a continuous infusion of the same solution (0 005–0 01 ml/kg body wt/min) After 60 min of equilibration and two control periods of about 20 min a continuous i v saline infusion of Ringer s solution (147 mEq Na /l) was started The infusion rate was 0 2? ml/kg body wt/min and it was continued until 30 ml/kg body wt or 3% of the body weight had been infused During saline infusion urine was collected during 15 minute periods Inulin in blood and urine was estimated by the anthron method (8) and PAH by the method described by Smith et al (15) Sodium was determined with an Eppendorff flame photometer

Statistical analysis

Two way analysis of variance was used for testing differences between investigations carried out at different times during the one year follow up The regression coefficient was calculated and used to measure the increase of sodium excretion during isotonic saline volume expansion Correlation coefficients were invariably higher than 0 8 From the analysis of variance linear comparisons were made Data are presented as means ± S E M

Five children with previous cystitis but without signs of renal involvement were used as controls for urinary sodium excretion during isotonic saline infusion Reported findings for GFR C_{PAH} and filtration fraction (FF) in healthy young adults were used as reference values (1)

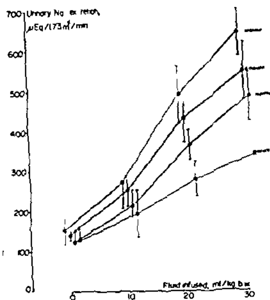


Fig. 3a. The urinary Na excretion related to BSA in post-streptococcal GN during continuous isotonic saline infusion (147 mEq Na /l 0 ? ml/kg body wt/min until 3% of the body wt was infused) shortly after the onset of the disease and two, six and 1 months later. Closed circles and bars correspond to means and standard error of the mean (S.E.M.). The shaded area shows the range of values in controls (i.e. children with previous cystitis but without signs of renal involvement).

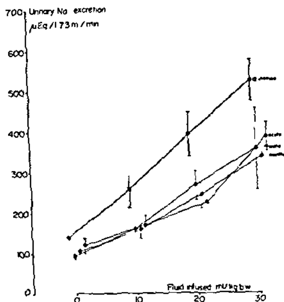


Fig. 3b. The urinary Na excretion related to BSA in IgA GN during continuous isotonic saline infusion (147 mEq Na /l 0 ?? ml/kg body wt/min until 3% of the body wt was infused) shortly after the onset of the disease and two, six and 1 months later. Closed circles and bars correspond to means and S.E.M. The shaded area indicates the range of values in control.

creased to normal levels on the last examination. The quotient of GFR and C_{PAH}, i.e. the filtration fraction (FF), is generally used as an index of the fraction of fluid that is filtered from the plasma passing the glomeruli. Immediately after the onset of the disease the FF during HP in PS GN was decreased and invariably lower than in IgA GN (Table 2). Thereafter the FF was normal in both disorders.

In the patient with non-streptococcal proliferative GN, the GFR during HP (Fig. 1a) was depressed on the first study shortly after the onset of the disease but it had normalized two months later. After 12 months, the GFR on the other hand, was again depressed, but during VE normalization took place. FF during HP was low on the first investigation as well as 12 months later, and VE influenced FF only slightly (Table 2).

Sodium excretion

The renal control of sodium excretion was evaluated by determining the natriuretic response to an isotonic VE with saline. Normally urinary sodium excretion increases in a characteristic manner during saline infusion (see controls in Figs. 3a, 3b). The changes in the natriuretic response to VE were similar in PS and IgA GN. Sodium excretion as related to body surface area or glomerular filtration was lower on the first examination than 12 months later (Table 3, Figs. 3a, 3b). The differences were significant for both in PS GN ($p<0.05$), but only for sodium excretion related to body surface area in IgA GN ($p<0.05$). In PS GN there appeared to be a progressive increase in the capacity to excrete sodium during the year, while in IgA GN the capacity to excrete sodium remained depressed at least during the first six months, but it then increased to normal levels.

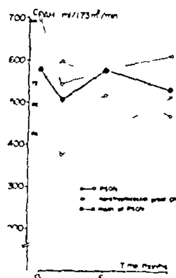


Fig 2a The C_{PAH} during HP in PS and non streptococcal proliferative GN shortly after the onset of the disease and two six and 12 months later The means (closed circles) and individual values (open circles) in *PS GN* (continuous connecting lines) and non streptococcal proliferative GN (interrupted connecting line) are shown

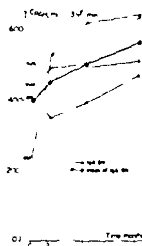


Fig 2b The C_{PAH} during HP in IgA GN shortly after the onset of the disease and two six and 12 months later The means (closed circles) and individual values (open circles) are shown

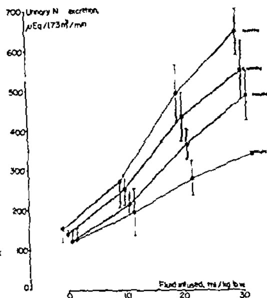
The first biopsy from the patient classified as non streptococcal proliferative GN (P A) showed diffuse glomerular hypercellularity IFL examination was positive only for C3 which was distributed in a granular pattern along the glomerular basement membranes and in the mesangial regions After 12 months only a slight segmental increase in mesangial matrix remained but IFL examination was unchanged

Renal function

Hemodynamic studies GFR and C_{PAH} were determined at each study during hydropenia (HP) and during isotonic saline volume expansion (VE) In PS as well as in IgA GN the GFR during HP was depressed and significantly lower immediately following the onset of the disease than on subsequent studies ($p<0.01$) (Table 1) Serial changes in GFR during HP are illustrated in Fig 1*a* and 1*b* It is apparent that the main increase in GFR occurred during the first two months of the disease It is noteworthy that the depression of GFR in acute PS GN as well as in acute IgA GN was abolished by VE (Table 1) PS GN differed from IgA GN with regard to the effect on renal plasma flow In PS GN C_{PAH} during HP (Fig 2*a*) was normal on the first examination as well as later In IgA GN on the other hand the C_{PAH} during HP (Fig 2*b*) was reduced on the first examination but in

Table 2 *Filtration fraction (%) in acute glomerulonephritis*
Values are mean ± S E M HP=hydropenia VE=volume expansion

	Onset		12 months later		Difference onset – 12 months later	
	HP	*VE*	*HP*	*VE*	*HP*	*VE*
Poststreptococcal GN	12 6±0 7	14 8±0 5	19 2±1 8	23 9±1 6	+6 6 *NS* ($p<0.1$)	+9 1 $p<0.01$
IgA GN	19 9±2 5	21 9±4 9	16 9±0 9	20 2±0 5	−3 0 *NS*	−1 7 *NS*
Non streptococcal prolif GN	16 0	15 7	11 7	14 8	−4 3	−0 9



g 3a The urinary Na excretion related to BSA in post reptococcal GN during continuous isotonic saline infu on (147 mEq Na /l 0 2 ml/kg body wt/min until 3% the body wt was infused) shortly after the onset of the sease and two six and 1 months later Closed circles id bars correspond to means and standard error of the ean (S E M) The shaded area hows the range of val es in control (i e children with previous cystitis but ithout signs of renal involvement)

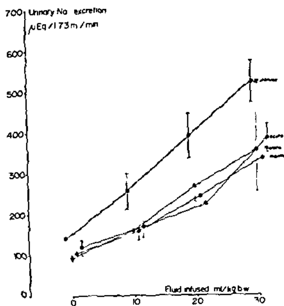


Fig 3b The urinary Na excretion related to BSA in IgA GN during continuous i otonic saline infusion (147 mEq Na /l 0 2 ml/kg body wt/min until 3% of the body wt was infused) shortly after the onset of the disease and two six and 1 months later Closed circles and bars correspond to means and S E M The shaded area indi cates the range of values in controls

.reased to normal levels on the last examina ion The quotient of GFR and C_{PAH} i e the filtration fraction (FF) is generally used as an index of the fraction of fluid that is filtered from the plasma passing the glomeruli Im mediately after the onset of the disease the FF during HP in PS GN was decreased and in variably lower than in IgA GN (Table 2) Thereafter the FF was normal in both dis orders

In the patient with non streptococcal pro- liferative GN the GFR during HP (Fig 1a) was depressed on the first study shortly after the onset of the disease but it had normalized two months later After 12 months the GFR on the other hand w is again depressed but during VE normalization took place FF dur ing HP was low on the first investigation as well as 12 months later and VE influenced FF only slightly (Table 2)

Sodium excretion

The renal control of sodium excretion was evaluated by determining the natriuretic re sponse to an isotonic VE with saline Nor mally urinary sodium excretion increases in a characteristic manner during saline infusion (see controls in Figs 3a 3b) The changes in the natriuretic response to VE were similar in PS and IgA GN Sodium excretion as related to body surface area or glomerular filtration was lower on the first examination than 12 months later (Table 3 Figs 3a 3b) The dif ferences were significant for both in PS GN ($p<0.05$) but only for sodium excretion re lated to body surface area in IgA GN ($p<0.05$) In PS GN there appeared to be a progressive increase in the capacity to excrete sodium dur ing the year while in IgA GN the capacity to excrete sodium remained depressed at least during the first six months but it then in creased to normal levels

Table 3. *Urinary Na excretion (μEq/min) after volume expansion in acute glomerulonephritis*
Values are mean ± S.E.M. BSA = related to 1.73 m² body surface area. GF = related to 100 ml glomerular filtrate

	Onset		12 months later		Difference onset 12 months later	
	BSA	GF	BSA	GF	BSA	GF
Poststreptococcal GN	336.8 ± 1.05	380.3 ± 54.1	638.5 ± 56.7	633.8 ± 42.6	+301.7 $p<0.05$*	+253.5 $p<0.05$
IgA GN	380.8 ± 29.8	367.9 ± 52.8	519.7 ± 50.5	508.8 ± 63.7	+138.9 $p<0.05$	+140.9 NS, <0
Non streptococcal prolif. GN	377.3	411.7	288.1	282.8	−89.2	−1.89

In the patient with non streptococcal proliferative GN, however, sodium excretion during isotonic saline VE was still depressed 12 months after the onset of the disease (Table 3).

DISCUSSION

Eight patients were admitted to this study. Three had PSGN, four IgA GN and one non streptococcal proliferative GN. Although the series is small it reflects the commonest causes of childhood GN. PS GN is probably the most common type of GN. The incidence of this disease, however, seems to be declining in Sweden. IgA GN has been reported to account for 12% of biopsied glomerulopathies in children (12). All patients in the present study classified as IgA GN showed the characteristic episodes of macroscopic hematuria in association with upper respiratory tract infections, together with IgA predominantly in mesangial regions (12, 13). The first episode of macroscopic hematuria was, however, more intensive than the later ones and in some aspects its manifestations closely resembled those of the acute phase of classical GN, suggesting that the patients were followed from the onset of their disease.

The pathophysiology of IgA GN resembled in many respects the pathophysiology of PS GN during the first year after the onset of the disease. In both disorders the GFR was decreased at the onset and normal 12 months later. The capacity to excrete sodium was also reduced at the onset of both disorders and normal 12 months later. The GFR increased to a value that was about 90% of the mean in healthy individuals during hydropenia two months after the onset of both disorders. The early restitution of GFR accords with previously reported determinations of GFR in acute GN (4, 11). It is not clear why the patient with non streptococcal proliferative GN at 12 months follow up had a decreased GFR during HP. The prolonged C3 depression indicates delayed recovery. However, morphological improvement occurred and the GFR depression was abolished by VE (Table 1). This would suggest that the depression of GFR was due to temporary functional changes rather than irreversible structural changes.

The effect of acute PS GN and IgA GN on effective renal plasma flow differed. In IgA GN, the C_{PAH} was low at the onset and the GFR was reduced in proportion to the C_{PAH}. In PS GN, on the other hand, the C_{PAH} was normal at the onset and the GFR was reduced out of proportion to the C_{PAH}. One might therefore speculate that in acute IgA GN the GFR is reduced because of increased renal vascular resistance, while in acute PS GN the GFR is low because of reduced permeability of the glomerular capillary wall. It is of interest in this connection that in IgA GN the IFL-deposits are primarily confined to the mesangium, whereas in PS GN they to a greater part are located in or near the capillary basement membrane, which might imply a reduced permeability.

Decreased capacity to excrete an intrave

nous isotonic saline load was observed in all patients with acute GN The sodium excretion was reduced out of proportion to the glomerular filtration rate which suggests an enhanced tubular sodium reabsorption Enhanced tubular sodium reabsorption in conjunction with isotonic saline VE has previously been demonstrated in acute experimental GN in dogs (16) Except for slight edema for a few days in one patient with PS GN and slight transient hypertension in one patient with IgA GN our patients did not have overt signs of salt and water retention and no changes in blood pressure were later seen Recumbent renin aldosterone excretion blood volume and bromide space did not show any characteristic changes during the entire follow up period It seems likely however that the relative inability to increase sodium excretion during VE observed in this study reflects the disturbances which cause the increased extracellular volume that are characteristic of severer forms of acute GN

All studied parameters of renal function were normal 12 months after the onset of acute PS GN and IgA GN The late effects of PS GN and IgA GN on renal function can not yet be predicted A depressed GFR below 70 ml/1 73 m² BSA/min has been found in more than 70% of cases one to 16 years after the onset of PS GN (2) It is therefore possible that a normal GFR 12 months after the onset of PSGN and IgA GN does not reflect a complete recovery but rather a functional adaptation to the disease It is apparent from this study that renal functional changes do not necessarily parallel renal structural changes and clinical signs of glomerular disease All patients with IgA GN after one year had clinical signs of active glomerular disease i e microscopic hematuria and episodes of macroscopic hematuria They also had residual structural glomerular changes and a positive IFL at the end of the follow up period The biopsy changes were about the same as at the onset Renal function however was within normal limits on the 12 month follow up study This suggests that renal functional adaptation occurs after the onset of acute GN If the disease progresses and further structural changes occur a normal GFR is no longer attainable Subsequent deterioration in renal function would probably then occur in the course of GN

ACKNOWLEDGEMENTS

This study was supported by grants from the Swedish Medical Research Council (B76-19X 7049 10) and from the Research Funds of Karolinska Institute

REFERENCES

1 Aperia A Broberger O Ericsson N O & Feychting H Renal function in man during transition from hydropenia to water diuresis with superimposed mannitol load Acta Physiol Scand 77 479 1969
2 Baldwin D S Poststreptococcal glomerulonephritis a progressive disease? Am J Med 62 1 1977
3 Berg R Bergstrand A Bergstrom J Cunningham R Tagesson Ch & Wasserman J Rapidly progressive glomerulonephritis with antibodies against glomerular basement membranes in serum and kidneys Clin Nephrol 5 37 1976
4 Earle D P Farber S J Alexander J D & Pellegrino E D Renal function and electrolyte metabolism in acute glomerulonephritis J Clin Invest 30 421 1951
5 Finley S C & Hare R S Bromide space in infants and children Am J Dis Child 98 749 1959
6 Gurland H J Brunner F P Chantler C Jacobs C Scharer K Selwood N H Spies G & Wing A J Combined report on regular dialysis and transplantation in Europe VI In B H B Robinson P Vereerstraeten & J B Hawkins (eds) Proceedings of the European Dialysis and Transplant Association Vol 13 Pitman Press Bath 1976 p ?
7 Haber E Koerner T Page L B Kliman B & Purnode A Application of a radioimmunoassay for angiotensin I to the physiologic measurements of plasma renin activity in normal human subjects J Clin Endocrinol 29 1349 1969
8 Hilger H H Klumper J D & Ullrich K J Wasserruckresorption und Ionentransport durch die Sammelrohrzellen der Saugetierniere Pflugers Arch 267 18 1958
9 Kliman B & Peterson R E Double isotope derivative assay of aldosterone in biological extracts J Biol Chem 235 1639 1960
10 Langan J Jackson R Adlin E V & Channick B J A simple radioimmunoassay for urinary aldosterone J Clin Endocrinol Metab 38 189 1974
11 Lewy J Cruz H & Metcoff J Acute renal failure and some renal functions in acute glomerulonephritis In J Metcoff (ed) Acute glomerulonephritis Little Brown & Co Boston 1967 p 339

12 Levy M Beaufils H Gubler M C & Habib R Idiopathic recurrent microscopic hematuria and mesangial IgA–IgG deposits in children (Berger s disease) *Clin Nephrol 1* 63 1973
13 McEnery P T McAdams A J & West C D Glomerular morphology natural history and treatment of children with IgA–IgG mesangial nephropathy *In* P Kincaid Smith T H Mathew & E L Becker (eds) *Glomerulonephritis Morphology Natural history and Treatment* Wiley New York 1973 p 305
14 Scharer K Chantler C Brunner F P Gurland H J Jacobs C Selwood N H Spies G & Wing A J Combined report on regular dialysis and transplantation of children in Europe *In* B H B Robinson P Vereerstraeten & J B Hawkins (eds) *Proceedings of the European Dialysis and Transplant Association* Vol 13 Pitman Press Bath 1976 p 59
15 Smith H W Finkelstein N Aliminosa L Crawford B & Graber M The renal clearances of substituted hippuric acid derivatives and other aromatic acids in dog and man *J Clin Invest 24* 388 1945
16 Wagnild J P Gutmann F D & Imm B Functional adaptation of nephrons in dogs with acute progressing to chronic experimental glomerulonephritis *J Clin Invest 57* 1575 1976

Submitted May 2 1978
Accepted Aug 22 1978

(T L) Department of Pediatrics Karolinska Institutet
St Goran s Children s Hospital
Box 12500
S 11281 Stockholm
Sweden

Acta Pædiatr Scand 68 181–186 1979

STRUCTURAL AND FUNCTIONAL ABNORMALITIES OF THE SMALL INTESTINE IN INFANTS AND YOUNG CHILDREN WITH ROTAVIRUS ENTERITIS

G P DAVIDSON and G L BARNES

From the Department of Gastroenterology Royal Children s Hospital Parkville Victoria Australia

ABSTRACT Davidson G P and Barnes G L (Department of Gastroenterology Royal Children s Hospital Parkville Victoria Australia) Structural and functional abnormalities of the small intestine in infants and young children with rotavirus enteritis Acta Paediatr Scand 68 181 1979.—Structural and functional alterations in duodenal mucosa from 17 children with rotavirus enteritis were assessed Structural changes were found in specimens from all patients Patients with the most severe mucosal damage were more likely to require intravenous therapy to correct dehydration Depression of one or more mucosal disaccharidases was found in 14 of 16 patients Repeat duodenal biopsy three to eight weeks later in six patients showed marked improvement The study clearly shows that rotavirus can cause a marked structural and functional lesion in the upper small intestine which is rapidly reversible

KEY WORDS Acute enteritis children diarrhoea disaccharidases rotavirus duodenal biopsy

Several studies have reported the presence of *structural* abnormalities in duodenal mucosa of children with acute gastroenteritis (3 16) In 1973 studies from this Department showed abnormalities of the duodenal mucosa in 26 of 31 infants with acute gastroenteritis (3) Abnormally low mucosal disaccharidase levels were found in 16 of the infants A recognized bacterial pathogen was isolated from only one patient In the remainder it was presumed that the causative agent was a virus although *C albicans* may have been important in some patients (4)

Later in the year Bishop et al identified a specific virus as the cause of acute non bacterial enteritis in a group of children admitted to the same hospital (5 6) This virus has not yet been fully characterized and many names have been assigned to it including orbivirus (6) rotavirus (9) duovirus (8) reovirus like agent (10) and infantile gastroenteritis virus (13) Rotavirus (the name most favoured) is now recognized as a major cause of acute infectious diarrhoea among infants and children throughout the world (14)

We report here structural and functional abnormalities of the duodenal mucosa in children with acute enteritis known rather than assumed to be caused by rotavirus infection

PATIENTS AND METHODS

Twenty children aged from two to 33 months with acute non bacterial enteritis were examined and had a small bowel biopsy All were admitted to the Royal Children s Hospital Melbourne during April to August 1973 Acute enteritis was defined as a febrile illness of less than 10 days duration associated with diarrhoea and vomiting where there was no other evident cause for the symptoms Rotavirus infection was identified in 17 of these patients (13 males and four females) and they form the subject of this report Patients one to nine and 11 to 14 in Table 1 were the subjects of earlier reports when rotavirus was first recognised (6 7)

Duodenal biopsy was performed 4 to 1 0 hours after onset of symptoms of acute enteritis (Table 1) A repeat biopsy was performed three to eight weeks later in six of the children (patients 2 3 4 8 9 1?) The biopsy procedure is described in detail elsewhere (17) The biopsy and its possible complications were explained to the parents as reported previously (6) Preparation of biopsy tissue for light and electron microscopy was as described (6) Preparation and examination of faecal specimens by electron microscopy was as previously described (7) Disaccharidase estimations were performed as before (3) Structural changes in duodenal mucosal tissue on light

Table 1 *Structural changes disaccharidase levels and electron microscope findings in l[…] children with rotavirus enteritis*

0=rotavirus negative += scanty ++=moderate +++=profuse NT=not tested

Patient	Age (mo)	Structural damage	Disaccharidases (units/g wet wt) Maltase (normal ≥9.0)	Sucrase (normal ≥3.5)	Lactase (normal ≥1.0)	Virus in epithelial cells	Virus in faeces	Duration of symptoms before biops[y] (hrs)
1	30	Severe	3.4	0.8	0.2	+++	+++	66
2	33	Severe	2.4	0.7	0.2	0	+	96
3	31	Moderate	2.0	0.3	0	+++	NT	36
4	8	Moderate	NT	NT	NT	+++	+++	[illegible]
5	19	Moderate	6.8	1.7	0.4	0	+	96
6	13	Moderate	5.6	1.2	0.3	++	NT	72
7	4	Moderate	10.6	2.7	1.0	+	+++	72
8	8	Moderate	10.1	2.4	2.8	+	+++	120
9	10	Moderate	5.9	1.7	0.4	0	+	120
10	6	Mild to moderate	13.3	4.0	1.7	++	++	96
11	4	Mild	9.9	4.2	0.3	+	+	96
12	14	Mild	2.0	0.3	0.3	++	+	84
13	11	Mild	6.0	1.7	0.9	+	+	24
14	22	Mild	10.0	3.0	1.6	+++	+++	24
15	11	Mild	5.5	1.5	0.9	+	+	96
16	18	Mild	13.1	3.5	1.9	+++	+	72
17	2	Mild	8.1	[illegible]	0.6	0	+	96

microscopy were graded as showing normal appearance mild moderate or severe change as described by Townley et al (18) Briefly mild changes included broadening of villi mild cellular infiltration of the lamina propria and early epithelial cell damage moderate change involved considerable blunting of villi obvious increase in inflammatory cells in the lamina propria and epithelial damage severe change showed complete villous flattening heavy cellular infiltration and severe epithelial damage

In 13 of 17 patients rotavirus particles were seen by electron microscopy of ultra thin sections of duodenum and in the other four rotavirus was identified by electron microscopy of faeces (Table 1)

RESULTS

Structural abnormalities were found in the duodenal mucosa of all patients with rotavirus enteritis In seven patients the duodenal mucosa was mildly abnormal Biopsies from eight patients showed moderate damage with blunting of villi increased crypt depth flattening of epithelial cells and an increase in inflammatory cells in the lamina propria (Fig 1) In two patients there were severe changes with loss of villi crypt hypertrophy cuboidal epithelium and marked inflammatory infiltrate (Fig 2)

The appearances were similar to those seen [in] coeliac disease although abnormalities tend[ed] to be patchy Electron microscopy of du[o]denal mucosa from two patients with seve[re] structural change (Fig 2) revealed mark[ed] changes and the presence of rotavirus particl[es] in the cytoplasm (Fig 3) Three weeks later repeat duodenal biopsy in one of these p[a]tients showed normal mucosal structure [by] light (Fig 4) and electron microscopy (Fig [5])

Disaccharidase levels are listed in Table [1] Depression of one or more enzymes was foun[d] in 14 of 16 specimens The biopsy specim[en] from patient 4 was too small for measureme[nt] of disaccharidase levels In 10 all three e[…]

Fig 1 Low magnification of biopsy from patient with r[o]tavirus enteritis showing moderate structural damag[e] Villi are blunted and widened crypt depth is increase[d] epithelial cells are abnormal and there is an obvious [in]crease in inflammatory cells in lamina propria (haem[a]toxylin and eosin ×40)

Fig 2 Low magnification of section of duodenal mu[c]o[sa] from patient with rotavirus enteritis showing severe stru[c]tural damage with loss of villi crypt hypertrophy e[pi]thelial cell damage and marked increase in inflammato[ry] cells in the lamina propria (haematoxylin and eosin ×4[0])



1

2

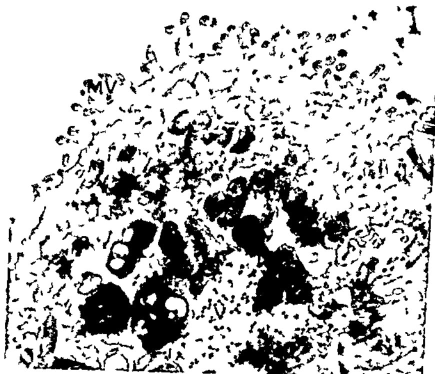


Fig. 3 Electron micrograph of apical portion of epithelial cell from biopsy in Fig 1. Microvilli (*MV*) are severely damaged, mitochondria (*M*) are swollen and numerous virus particles (*V*) are aggregated within distended endoplasmic reticulum cisterna (×20000)

zymes were low. Only two patients had normal levels of all disaccharidases.

Six children had a second biopsy. In four of them (patients 2, 3, 9, 12) disaccharidase levels were initially very low, resembling the pattern seen in coeliac disease. To exclude this diagnosis duodenal biopsy was repeated three to eight weeks later. The specimen from patient 3 was too small to allow disaccharidase assay but duodenal structure had returned to normal. Specimens from the other three patients had normal structure and enzyme levels. Two patients (patients 4 and 8) continued to have diarrhoea following discharge from hospital and a repeat duodenal biopsy was performed to exclude underlying disease. Mucosal structure had recovered but the disaccharidase levels suggested relative sucrase depression (2, 12). Both patients responded symptomatically to a low sucrose diet.

DISCUSSION

Structural and functional alteration of the duodenal mucosa in these 17 children with proven rotavirus enteritis was similar to that reported in our earlier series of 31 children in whom the cause of the acute enteritis was not identified (3) (Table 2). In retrospect rotavirus was probably responsible for the diarrhoeal illness in most of those children as only one bacterial pathogen was isolated (3).

The damage observed in rotavirus enteritis can be severe enough to be confused with the structural appearance seen in coeliac disease. It appears that the mucosal damage caused by rotavirus can be rapidly repaired as biopsies repeated as early as three weeks after onset of symptoms were normal. The mucosal damage in acute gastroenteritis is quite variable in degree and may be patchy as noted by others (3). Although the numbers are small, children with a more severe mucosal lesion may be more likely to be dehydrated and require intravenous therapy for rehydration (nine of 10 with moderate or severe damage compared with four of seven with mild structural damage).

Diminished disaccharidase activity in duodenal mucosal homogenates from the majority of patients in this study suggested that sugar malabsorption might have been a significant problem during the acute phase of rotavirus enteritis. This was not so. Stool clinitests

Fig 4 Low magnification of biopsy from patient shown in Fig 1 three weeks after recovery from rotavirus enteritis showing normal duodenal mucosa (haematoxylin and eosin ×40)

were rarely positive (11) but this may be explained by the fact that most infants were drinking only glucose containing fluids at the time of study The earlier study by Barnes et al (3) suggested that prolonged sugar intolerance was age related and not predictable by acute disaccharidase depression Only four of the 17 children in the present study were less than six months of age so the majority would not have been expected to exhibit prolonged sugar intolerance

As rotavirus enteritis can now be effectively diagnosed by faecal electron microscopy or serology it is not likely that many children in the future will have small bowel biopsies during the acute stage of the illness Structural damage to the upper small bowel mucosa has been described in adults infected with other viruses including the Norwalk (1) and Hawaii (15) agents small picorna/parvolike viruses which to date have not been associated with infectious diarrhoea in infants Similarly mu



Fig 5 Electron micrograph of representative area from biopsy shown in Fig 4 showing apical portion of two normal enterocytes with microvilli junctional complex (JC) and mitochondria (M) (× 0000)

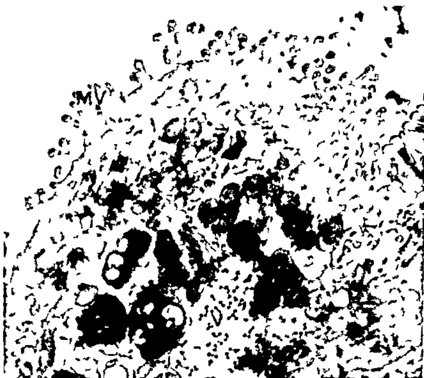


FIG 3 Electron micrograp[h] tion of epithelial cell from biopsy in ro[tavirus] Microvilli (MV) are severely dama[ged] mitochondria (M) are swollen and nu[mer]ous virus particles (V) are aggregated w[ithin] distended endoplasmic reticulum cistern (×20000)

zymes were low Only two patients had normal levels of all disaccharidases

Six children had a second biopsy In four of them (patients 2 3 9 12) disaccharidase levels were initially very low resembling the pattern seen in coeliac disease To exclude this diagnosis duodenal biopsy was repeated three to eight weeks later The specimen from patient 3 was too small to allow disaccharidase assay but duodenal structure had returned to normal Specimens from the other three patients had normal structure and enzyme levels Two patients (patients 4 and 8) continued to have diarrhoea following discharge from hospital and a repeat duodenal biopsy was performed to exclude underlying disease Mucosal structure had recovered but the disaccharidase levels suggested relative sucrase depression (2 12) Both patients responded symptomatically to a low sucrose diet

DISCUSSION

Structural and functional alteration of the duodenal mucosa in these 17 children with proven rotavirus enteritis was similar to that reported in our earlier series of 31 children in whom the cause of the acute enteritis was not identi[-] fied (3) (Table 2) In retrospect rotavirus w[as] probably responsible for the diarrhoeal illne[ss] in most of those children as only one bacteri[al] pathogen was isolated (3)

The damage observed in rotavirus enteri[tis] can be severe enough to be confused with t[he] structural appearance seen in coeliac diseas[e] It appears that the mucosal damage caused [by] rotavirus can be rapidly repaired as biopsi[es] repeated as early as three weeks after onset [of] symptoms were normal The mucosal dama[ge] in acute gastroenteritis is quite variable [in] degree and may be patchy as noted by othe[rs] (3) Although the numbers are small childr[en] with a more severe mucosal lesion may [be] more likely to be dehydrated and require int[ra-] venous therapy for rehydration (nine of [ten] with moderate or severe damage compar[ed] with four of seven with mild structural da[m-] age)

Diminished disaccharidase activity in du[o-] denal mucosal homogenates from the major[ity] of patients in this study suggested that sug[ar] malabsorption might have been a significa[nt] problem during the acute phase of rotavir[us] enteritis This was not so Stool clinite[st]

Acta Pædiatr Scand 68 187–191 1979

AGAMMAGLOBULINAEMIA ASSOCIATED WITH THE OCCURRENCE OF A MONOCLONAL IMMUNOGLOBULIN

G F M HENDRICKX B J M ZEGERS and J W STOOP

From the Department of Immunology University Children s Hospital Het Wilhelmina Kinder iekenhuis Utrecht The Netherlands

ABSTRACT Hendrickx G F M Zegers B J M and Stoop J W (University Children s Hospital Het Wilhelmina Kinderziekenhuis Utrecht The Netherlands) Agammaglobulinaemia associated with the occurrence of a monoclonal immunoglobulin Acta Paediatr Scand 68 187 1979.—A patient is described with a monoclonal immunoglobulin of the IgG class in the serum and no detectable IgM and IgA Extensive immunological investigations showed the absence of B-lymphocytes in bone marrow and peripheral blood Moreover plasma cells were not present in the bone marrow The monoclonal IgG was synthesized in the gastrointestinal tract The cellular immune status of the patient was normal Clinically the patient suffered from gastrointestinal and severe respiratory tract infections It was concluded that the findings are consistent with the diagnosis congenital agammaglobulinaemia with concurrence of monoclonal IgG It was postulated that the cell clone in the gastrointestinal tract resulted from an escape of a pre B cell clone from the recognized arrest of pre-B cells in congenital agammaglobulinaemia

KEY WORDS Agammaglobulinaemia monoclonal immunoglobulin

Patients with primary agammaglobulinaemia can be divided into two groups one with the congenital type and the other with the late onset type of disease (8 11) The first of these groups comprises patients with an X linked recessive genetic pattern or an autosomal recessive pattern as well as isolated cases without a hereditary factor (8) The clinical picture is characterized by an extreme susceptibility to bacterial infections The serum of these patients contains less than 2 g/l IgG and usually lacks IgA and IgM No (or very few) immunoglobulin bearing B lymphocytes are found in the peripheral blood and no (or very few) plasma cells in the bone marrow (1 4) Plasma cells have also been reported to be absent in the (intestinal) mucosa of patients with an X linked agammaglobulinaemia (?)

A distinct genetic pattern is not known for primary acquired agammaglobulinaemia which is also called late-onset agammaglobulinaemia (8) The signs and symptoms usually appear after the age of ten years The immunoglobulin levels in the serum are usually higher than in the congenital forms B lymphocytes are present in the peripheral blood and also in the bone marrow usually in normal numbers (4 11) Some of the patients show plasma cells in the intestinal mucosa (15) Patients with the late-onset agammaglobulinaemia have not only an increased susceptibility to bacterial infections but also a greater chance of developing autoimmune diseases and malignant processes (11)

The occurrence of monoclonal immunoglobulins in childhood is extremely rare (5) The case reported here concerns a child with a severe immunoglobulin deficiency in whom a monoclonal IgG was demonstrated

MATERIALS AND METHODS

Humoral and cellular immunity of the patient has essentially been investigated as described in detail earlier (?8) Membrane immunofluorescence of the B lymphocytes and the cytoplasmic immunofluorescence of the plasma cells in the bone marrow has been done as described by Vossen & Hijmans (?7) Cytoplasmic immunofluorescence of the jejunal and rectal biopsies was performed as described by Mul et al (16) The anal

Table 2 *Comparison of mucosal structure and disaccharidase levels from two groups of children with acute gastroenteritis*

	Barnes et al 1973(1) (unknown aetiology)	Present study (rotavirus enteritis)
Mucosal structural changes		
Normal	5	0
Mild change	11	8
Moderate change	10	7
Severe change	5	2
Disaccharidase depression (1 or more)	16	14
Total patients	31	17

Disaccharidase levels not measured in patient 4

cosal disaccharidase depression a prominent feature in rotavirus enteritis has also been described in other viral enteritides (1 15) This report documents structural and functional changes in small bowel mucosa in a group of children known to be suffering from rotavirus enteritis

ACKNOWLEDGEMENTS

The authors acknowledge the support and encouragement of Dr R R W Townley whose patients we studied and whose initiative prompted this work We also thank Dr Ruth F Bishop who conceived the idea of applying the technique of electron microscopy to the study of gastroenteritis which ultimately led to the discovery of the rotavirus We thank Mr Brian Ruck for the electron micrographs Mr J Smith for the light microscopy photographs Mrs R Kay for preparation of the histological sections and Mr Max Murray for disaccharidase assays

REFERENCES

1 Agus S G Dolin R Wyatt R G Tousimis A J & Northrup R S Acute infectious non bacterial gastroenteritis intestinal histopathology Histologic and enzymatic alterations during illness produced by the Norwalk agent in man *Ann Intern Med* 79 18 1973
2 Ament M E Perera D R & Esther L J Sucrase isomaltase deficiency—a frequently misdiagnosed disease *J Pediatr* 83 721 1973
3 Barnes G L & Townley R R W Duodenal mucosal damage in 31 infants with gastroentera *Arch Dis Child* 48 343 1973
4 Bishop R F Barnes G L & Townley R R W Microbial flora of stomach and small intestine in fantile gastroenteritis *Acta Paediatr Scand* 63 41 1974
5 Bishop R F Davidson G P Holmes I H Ruck B J Evidence for viral gastroenteritis N E *J Med* 289 1096 1973
6 Bishop R F Davidson G P Holmes I H Ruck B J Virus particles in epithelial cells of du denal mucosa from children with acute non bacter gastroenteritis *Lancet II* 1281 1973
7 Bishop R F Davidson G P Holmes I H Ruck B J Detection of a new virus by elect microscopy of faecal extracts from children w acute gastroenteritis *Lancet I* 149 1974
8 Davidson G P Bishop R F Townley R R W Holmes I H & Ruck B J Importance of a ne virus in acute sporadic enteritis in children *Lance* 242 1975
9 Flewett T H Bryden A S Davies H Wood G N Bridger J C & Derrick J M Relation b tween viruses from acute gastroenteritis of childr and newborn calves *Lancet II* 61 1974
10 Kapikian A Z Kim H W Wyatt R G Rod guez W J Cline W L Parrott R H & Ch nock R M Reoviruslike agent in stools Associa with infantile diarrhoea and development of serol tests *Science* 185 1049 1974
11 Kerry K R & Anderson C M A ward test f sugar in faeces *Lancet I* 981 1964
12 Kerry K R & Townley R R W Genetic a pe of intestinal sucrase isomaltase deficiency Au *Paediatr J* 1 223 1965
13 Martin M J Palmer E L & Middleton P J Ultrastructure of infantile gastroenteritis virus *Vir logy* 68 146 1975
14 Rotavirus of man and animals *Lancet I* 257 19
15 Schreiber D S Blacklow N R & Trier J S The small intestinal lesion induced by Hawaii age acute infectious non bacterial gastroenteriti *J Inf Dis* 129 705 1974
16 Tocalino H Licastro R Cardo A G & Wi liams M Histologic changes in the small intest in children with acute diarrhoea *J Paediatr* 78 90 1971
17 Townley R R W & Barnes G L Intestinal biop in childhood *Arch Dis Child* 48 480 1973
18 Townley R R W Khaw K T & Shwachman H Quantitative assay of disaccharidase activities small intestinal mucosal biopsy specimens in infan and childhood *Pediatrics* 36 911 1965

Submitted July 3 1978
Accepted Sept 19 1978

(G L B) Department of Gastroenterology
Royal Children s Hospital
Flemington Road
Parkville Victoria
Australia 3052

ıormal (62 %) In vitro stimulation with phyto ıaemagglutinin (PHA) pokeweed mitogen PWM) and tetanus toxoid (an anamnes ıc antigen) gave normal ^{14}C thymidine in orporation In mixed lymphocyte culture (MLC) experiments a normal stimulator ınd responder capacity was observed PWM stimulation of the patient s lymphocytes gave good lymphocyte proliferation as judged from the normal ^{14}C thymidine incorporation but blasts containing immunoglobulin were not demonstrable (9)

CLINICAL COURSE

already mentioned the *Giardia lamblia* infection was ce sfully treated before the investigations mentioned ove After the studies gammaglobulin substitution was ırted (initial dose 1 4 ml/kg of a 16 % solution followed a monthly maintenance dose of 0 7 ml/kg) The in ction of the respiratory tract was treated with an anti otic (amoxycillin) Moderate swelling of the mucosa of e sinus maxillare persisted The physical and radio- gi al signs in the lungs largely disappeared The lung nction improved Prophylaxis with antibiotics was owever required because symptoms and signs of bron pneumonic infect on reappeared after the amoxycillin a withdrawn The malabsorption was still present to the ıme degree six months after the treatment was started nd also after the disappearance of the *Gia d a lamblia* he increase in height and weight were normal and there ere no signs of nutritional deficits During this treat ent the serum IgG decreased to a level lower than 1 g/l as determined in serum obtained prior to each ammaglobulin injection The jejunum biopsy specimen aken after six months of treatment showed fewer plasma ell the illous atrophy was less pronounced and the acta e content (5 µmol/g wet weight) lay within normal mits

At present the patient has been followed for two years nd his condition is still good with an occasional exacer bation of the respir t ry tract infection which responded well to a therapeuti d sage of cotrimoxazol

DISCUSSION

The clinic il picture of this patient is consistent with a specific humoral defence disturbance The quantitative determination of the serum immunoglobulins (Ig) showed an IgG level that was not distinctly anomalous for the patient s age but IgM and IgA were not present This kind of serum Ig pattern is usually considered to indicate dysimmunoglobulinaemia Qualitative analysis of the IgG showed that it was monoclonal Specific antibodies were not demonstrable in the serum B lymphocytes were not present in the peripheral blood and neither these lymphocytes nor Ig containing plasma cells were present in the bone marrow The specific cellular immunity was intact Thus the findings in this case are more consistent with the diagnosis primary agammaglobulinaemia with—as a very striking feature—occurrence of monoclonal IgG

In children monoclonal Ig—sometimes transient—have been demonstrated for instance in some cases of (severe) combined immunodeficiency (4 19 24) secondary immunodeficiency and malignant processes of the lymphoreticular system (21 22 23) coeliac disease (18) the haemolytic uraemic syndrome (25) chromosomal abnormalities and congenital infections (4 17) Immunological reconstitution after bone marrow transplantation is often accompanied by the appearance in the serum of monoclonal Ig which are transient and reflect the maturation of the B cell system (20) To the best of our knowledge the clinical picture of multiple myeloma has never been described in childhood (12) and is also not to be expected in this age group (13) No indications pointing to multiple myeloma were found in our patient since the radiological investigation of the skeleton and the bone scintigraphy showed no anomalies the bone marrow was morphologically normal except for the absence of plasma cells Bence Jones protein was not found and there was no calciuria The production of monoclonal IgG was extra medullar and was localized in the gastrointestinal tract as shown by immunofluorescence studies in mucosal biopsy specimens Consequently the possibility of an extra-osseous myeloma or extra medullary plasmacytoma localized in the gastrointestinal tract could not be ruled out In these patients however abdominal complaints usually predominate but our patient did not have such complaints and neither the clinical nor the radiological investigation pro-

ysis of immunoglobulin containing blasts after culture of the lymphocytes with pokeweed mitogen (PWM) has been performed as published by Gmelig-Meyling et al. (9). The combined xylose–lactose tolerance test has been done as described by Fernandes & Van de Kamer (7). Gastrointestinal protein loss has been investigated by using intravenously administered $Cr^{51}Cl_3$. The small intestinal disaccharidases were analyzed as described by Van Eggermont & Hers (6).

CASE REPORT

R. J. was born at term after an uneventful pregnancy. Diphtheria, pertussis, tetanus, poliomyelitis and small pox vaccinations did not lead to unusual reactions. At the age of one and a half years, a severe *infection of* the respiratory tract required hospitalization and frequent recurrences were accompanied by a high fever. Chicken pox and measles ran an uncomplicated course, but mumps gave rise to a very severe herpetiform infection of the upper lip in association with a stomatitis ulcerosa. Quantitative determination of the serum immunoglobulins in this connection (at the age of five years) showed that neither IgA nor IgM were present in the serum. The IgG content was 5.31 g/l. Agar gel and immuno electrophoresis were not performed. At the age of seven, the child was referred to our hospital because of increasingly copious purulent sputum and suspicion of bronchiectasis. The height and weight lay at P90 and P70 for the age respectively. Rhinitis mucopurulenta was observed and at auscultation of the lungs diffusely spread rhonchi and crepitations were heard in both lower lobes. The teeth were carious. The physical examination showed no other anomalies.

The sedimentation rate of the erythrocytes was 25 mm. The haemoglobin and haematocrit values were normal. The blood count showed leucocytosis (21.3×10⁹/l); the differential count showed 1% eosinophils, 2% basophils, 4% bandforms, 79% segmental neutrophils and 14% lymphocytes. Normal serum levels were found for electrolytes, calcium, inorganic phosphate, alkaline phosphatase, urea, creatinine, transaminases, cholesterol and uric acid. The calcium content of the urine was normal. The sweat test gave normal results. The blood group was 0 rhesus positive. The total haemolytic complement content was elevated.

Radiological investigation of the skeleton and bone scintigraphy showed no anomalies. X-rays of the nasal sinuses showed sinusitis maxillaris, and the thorax X-ray showed infiltrative changes in both lower lobes. At bronchography, the right middle lobe bronchus was found to be narrowed with poor filling of the peripheral region. The branches of the right lower lobe bronchus showed irregular borders.

The lung function investigation showed a slightly reduced vital capacity, i.e. 1550 ml (normal for height 1800 ml). Culture of the nasal secretion and the sputum yielded an abundance of *Haemophilus influenzae*.

The faeces showed *Giardia lamblia*, which was treated with metronidazol before functional investigation of the gastrointestinal tract. Fat excretion was normal. Nitrogen excretion was, however, elevated (1.47 g/24 h; normal <1 g/24 h) (14). This could be partially ascribed to a protein loss, since in the $Cr^{51}Cl_3$ test the total excretion of the marker over a 4-day period amounted to 2.0% (normal <1%). D-xylose absorption was normal and *lactose uptake was reduced. The jejunum biopsy* specimen showed moderate and in some places even total atrophy of the villi as well as signs of chronic enteritis. The lactase level in the biopsy specimen was low (0.8 μmol/g wet weight). The levels of sucrase, isomaltase and maltase were normal.

IMMUNOLOGICAL INVESTIGATIONS

Humoral immunity

Agar gel electrophoresis of the serum showed a small homogeneous band in the gamma globulin region; on immuno electrophoresis with specific antisera this band proved to be IgG1, type λ. IgG of other subclasses or type κ was only present in very small amounts. The serum IgG content was 5.67 g/l. IgM and IgA were not demonstrable and the same *holds for isohaemagglutinins, antistaphy*lolysine, antistreptolysine and antibodies against the mumps and measles viruses. Antibodies against diphtheria, tetanus and poliomyelitis were also undemonstrable, even after a booster injection of DTP vaccine. No immunoglobulin bearing B lymphocytes were present in the peripheral blood or bone marrow, and no immunoglobulin containing plasma cells were *found in the latter*. Plasma cells found in the biopsy specimens from the jejunum and rectum contained IgG, type λ, but plasma cells with IgA or IgM were not present. In the nasal secretion IgG of type λ was found but not IgA or IgM. The urine did not contain Bence Jones protein.

Cellular immunity

The number of *lymphocytes in the peripheral* blood was always more than 2.0×10⁹/l. Delayed type hypersensitivity to mumps was present (*induration 15 mm*). After sensibilization with dinitrochlorobenzene (DNCB), the challenge led to a positive reaction. The percentage of T lymphocytes (E rosettes) was

normal (62%) In vitro stimulation with phytohaemagglutinin (PHA) pokeweed mitogen (PWM) and tetanus toxoid (an anamnestic antigen) gave normal ^{14}C thymidine incorporation In mixed lymphocyte culture (MLC) experiments a normal stimulator and responder capacity was observed PWM stimulation of the patient s lymphocytes gave good lymphocyte proliferation as judged from the normal ^{14}C thymidine incorporation but blasts containing immunoglobulin were not demonstrable (9)

CLINICAL COURSE

As already mentioned the *Giardia lamblia* infection was successfully treated before the investigations mentioned above After the studies gammaglobulin substitution was started (initial dose 1 4 ml/kg of a 16% solution followed by a monthly maintenance dose of 0 7 ml/kg) The infection of the respiratory tract was treated with an antibiotic (amoxycillin) Moderate swelling of the mucosa of the sinus maxillares persisted The physical and radiological signs in the lungs largely disappeared The lung function improved Prophylaxis with antibiotics was however required because symptoms and signs of bronchopneumonic infection reappeared after the amoxycillin wa withdrawn The malabsorption was still present to the same degree six months after the treatment was started and also after the disappearance of the *Giard a lamblia* The increase in height and weight were normal and there were no signs of nutritional deficits During this treatment the serum IgG decreased to a level lower than 0 3 g/l as determined in serum obtained prior to each gammaglobulin injection The jejunum biopsy specimen taken after six month of treatment showed fewer plasma cells the villous atrophy was less pronounced and the lactase content (5 μmol/g wet weight) lay within normal limits

At present the patient has been followed for two years and his condition is still good with an occasional exacerbation of the respiratory tract infection which responded well to a therapeutic dosage of cotrimoxazol

DISCUSSION

The clinical picture of this patient is consistent with a specific humoral defence disturbance The quantitative determination of the serum immunoglobulins (Ig) showed an IgG level that was not distinctly anomalous for the patient s age but IgM and IgA were not present This kind of serum Ig pattern is usually considered to indicate dysimmunoglobulinaemia Qualitative analysis of the IgG showed that it was monoclonal Specific antibodies were not demonstrable in the serum B lymphocytes were not present in the peripheral blood and neither these lymphocytes nor Ig containing plasma cells were present in the bone marrow The specific cellular immunity was intact Thus the findings in this case are more consistent with the diagnosis primary agammaglobulinaemia with—as a very striking feature—occurrence of monoclonal IgG

In children monoclonal Ig—sometimes transient—have been demonstrated for instance in some cases of (severe) combined immunodeficiency (4 19 24) secondary immunodeficiency and malignant processes of the lymphoreticular system (21 22 23) coeliac disease (18) the haemolytic uraemic syndrome (25) chromosomal abnormalities and congenital infections (4 17) Immunological reconstitution after bone marrow transplantation is often accompanied by the appearance in the serum of monoclonal Ig which are transient and reflect the maturation of the B cell system (20) To the best of our knowledge the clinical picture of multiple myeloma has never been described in childhood (12) and is also not to be expected in this age group (13) No indications pointing to multiple myeloma were found in our patient since the radiological investigation of the skeleton and the bone scintigraphy showed no anomalies the bone marrow was morphologically normal except for the absence of plasma cells Bence Jones protein was not found and there was no calciuria The production of monoclonal IgG was extra medullar and was localized in the gastrointestinal tract as shown by immunofluorescence studies in mucosal biopsy specimens Consequently the possibility of an extra osseous myeloma or extra medullary plasmacytoma localized in the gastrointestinal tract could not be ruled out In these patients however abdominal complaints usually predominate but our patient did not have such complaints and neither the clinical nor the radiological investigation pro-

ysis of immunoglobulin containing blasts after culture of the lymphocytes with pokeweed mitogen (PWM) has been performed is published by Gmelig Meyling et al (9) The combined xylose–lactose tolerance test has been done as described by Fernandes & Van de Kamer (7) Gastro intestinal protein loss has been investigated by using intravenously administered $Cr^{51}Cl_3$ The small intestinal disacchari dases were analyzed as described by Van Eggermont & Hers (6)

CASE REPORT

R L was born at term after an uneventful pregnancy Diphtheria pertussis tetanus poliomyelitis and small pox vaccinations did not lead to unusual reactions At the age of one and a half years a severe infection of the respiratory tract required hospitalization and frequent recurrences were accompanied by a high fever Chicken pox and measles ran an uncomplicated course but mumps gave rise to a very severe herpetiform infection of the upper lip in association with a stomatitis ulcerosa Quantitative determination of the serum immunoglobulins in this connection (at the age of five years) showed that neither IgA nor IgM were present in the serum The IgG content was 5 31 g/l Agar gel and immuno electro phoresis were not performed At the age of seven the child was referred to our hospital because of increasingly copious purulent sputum and suspicion of bronchiectasis The height and weight lay at P90 and P70 for the age respectively Rhinitis mucopurulenta was observed and at auscultation of the lungs diffusely spread rhonchi and crepitations were heard in both lower lobes The teeth were carious The physical examination showed no other anomalies

The sedimentation rate of the erythrocytes was 25 mm The haemoglobin and haematocrit values were normal The blood count showed leucocytosis (21 3×10 /l) the differential count showed 1% eosinophils 2% basophils 4% bandforms 79% segmental neutrophils and 14% lymphocytes Normal serum levels were found for electro lytes calcium inorganic phosphate alkaline phosphatase urea creatinine transaminases cholesterol and uric acid The calcium content of the urine was normal The sweat test gave normal results The blood group was 0 rhesus positive The total haemolytic complement content was elevated

Radiological investigation of the skeleton and bone scintigraphy showed no anomalies X rays of the nasal sinuses showed sinusitis maxillaris and the thorax X ray showed infiltrative changes in both lower lobes At bronchography the right middle lobe bronchus was found to be narrowed with poor filling of the peripheral region The branches of the right lower lobe bronchus showed irregular borders

The lung function investigation showed a slightly re duced vital capacity i e 1550 ml (normal for height 1800 ml) Culture of the nasal secretion and the sputum yielded an abundance of *Haemophilus influenzae*

The faeces showed *Giardia lamblia* which was treated with metronidazol before functional investigation of the gastrointestinal tract Fat excretion was normal Nitrogen excretion was however elevated (1 47 g/ 4 h normal <1 g/24 h) (14) This could be partially ascribed to a protein loss since in the $Cr^{51}Cl_3$ test the total excretion of the marker over a 4-day period amounted to 2 0 % (normal <1%) D xylose absorption was normal and lactose uptake was reduced The jejunum biopsy speci men showed moderate and in some places even total atrophy of the villi as well as signs of chronic enteritis The lactase level in the biopsy specimen was low (0 8 µmol/g wet weight) The levels of sucrase isomaltase and maltase were normal

IMMUNOLOGICAL INVESTIGATIONS

Humoral immunity

Agar gel electrophoresis of the serum showed a small homogeneous band in the gamma globulin region on immuno electrophoresis with specific antisera this band proved to be IgG1 type λ IgG of other subclasses or type κ was only present in very small amounts The serum IgG content was 5 67 g/l IgM and IgA were not demonstrable and the same holds for isohaem agglutinins antistaphy lolysine antistreptolysine and antibodies against the mumps and measles viruses Anti bodies against diphtheria tetanus and polio myelitis were also undemonstrable even after a booster injection of DTP vaccine No im munoglobulin bearing B lymphocytes were present in the peripheral blood or bone mar row and no immunoglobulin containing plas ma cells were found in the latter Plasma cells found in the biopsy specimens from the jeju num and rectum contained IgG type λ but plasma cells with IgA or IgM were not present In the nasal secretion IgG of type λ was found but not IgA or IgM The urine did not contain Bence Jones protein

Cellular immunity

The number of lymphocytes in the peripheral blood was always more than 2 0×10⁹/l De layed type hypersensitivity to mumps was present (induration 15 mm) After sensibiliza tion with dinitrochlorobenzene (DNCB) the challenge led to a positive reaction The per centage of T lymphocytes (E rosettes) was

ormal (62 %) In vitro stimulation with phyto aemagglutinin (PHA) pokeweed mitogen PWM) and tetanus toxoid (an anamnes ic antigen) gave normal ^{14}C thymidine in :orporation In mixed lymphocyte culture MLC) experiments a normal stimulator and responder capacity was observed PWM stimulation of the patient s lymphocytes gave good lymphocyte proliferation as judged from the normal ^{14}C thymidine incorporation but blasts containing immunoglobulin were not demonstrable (9)

CLINICAL COURSE

As already mentioned the *Giardia lamblia* infection was successfully treated before the investigations mentioned above After the studies gammaglobulin substitution was started (initial dose 1 4 ml/kg of a 16 % solution followed by a monthly maintenance dose of 0 7 ml/kg) The in fection of the re piratory tract was treated with an anti biotic (amoxycillin) Moderate swelling of the muco a of the sinus maxillares persisted The physical and radio-logical signs in the lungs largely disappeared The lung function improved Prophylaxis with antibiotics was however required because symptoms and signs of bron ch pneumonic infection reappeared after the amoxycillin was withdrawn The malab orption was still present to the same degree six months after the treatment was started and also after the disappearance of the *Giardia lamblia* The increase in height and weight were normal and there were no signs of nutritional deficits During this treat ment the serum IgG decreased to a level lower than 0 3 g/l as determined in serum obtained prior to each gammaglobulin injection The jejunum biopsy specimen taken after six months of treatment showed fewer plasma cell the villous atrophy was less pronounced and the lactase content (5 µmol/g wet weight) lay within normal lim ts

At present the patient has been followed for two years and h s condition is st ll good with an occasional exacer bat n of the re piratory tract infection which responded well to a therapeutic dosage of c trimoxazol

DISCUSSION

The clinical picture of this patient is consistent with a specific humoral defence disturbance The quantitative determination of the serum immunoglobulins (Ig) showed an IgG level that was not distinctly anomalous for the patient s age but IgM and IgA were not present This [illegible] g pattern is usually considered [illegible] mmunoglobulinaemia Qualitative analysis of the IgG showed that it was monoclonal Specific antibodies were not demonstrable in the serum B lymphocytes were not present in the peripheral blood and neither these lymphocytes nor Ig containing plasma cells were present in the bone marrow The specific cellular immunity was intact Thus the findings in this case are more con sistent with the diagnosis primary agamma globulinaemia with—as a very striking fea ture—occurrence of monoclonal IgG

In children monoclonal Ig—sometimes transient—have been demonstrated for in stance in some cases of (severe) combined im munodeficiency (4 19 24) secondary im munodeficiency and malignant processes of the lymphoreticular system (21 22 23) coeliac disease (18) the haemolytic uraemic syndrome (25) chromosomal abnormalities and congenital infections (4 17) Immuno logical reconstitution after bone marrow transplantation is often accompanied by the appearance in the serum of monoclonal Ig which are transient and reflect the maturation of the B cell system (20) To the best of our knowledge the clinical picture of multiple myeloma has never been described in child hood (12) and is also not to be expected in this age group (13) No indications pointing to multiple myeloma were found in our patient since the radiological investigation of the skeleton and the bone scintigraphy showed no anomalies the bone marrow was morpho-logically normal except for the absence of plasma cells Bence Jones protein was not found and there was no calciuria The pro-duction of monoclonal IgG was extra medullar and was localized in the gastrointestinal tract as shown by immunofluorescence studies in mucosal biopsy specimens Consequently the possibility of an extra-osseous myeloma or extra medullary plasmacytoma localized in the gastrointestinal tract could not be ruled out In these patients however abdominal com plaints usually predominate but our patient did not have such complaints and neither the clinical nor the radiological investigation pro

ysis of immunoglobulin containing blasts after culture of the lymphocytes with pokeweed mitogen (PWM) has been performed as published by Gmelig Meyling et al (9) The combined xylose–lactose tolerance test has been done as described by Fernandes & Van de Kamer (7) Gastrointestinal protein loss has been investigated by using intravenously administered $Cr^{51}Cl_3$ The small intestinal disaccharidases were analyzed as described by Van Eggermont & Hers (6)

CASE REPORT

R L was born at term after an uneventful pregnancy Diphtheria pertussis tetanus poliomyelitis and small pox vaccinations did not lead to unusual reactions At the age of one and a half years a severe infection of the respiratory tract required hospitalization and frequent recurrences were accompanied by a high fever Chicken pox and measles ran an uncomplicated course but *mumps gave rise to a very severe herpetiform infection* of the upper lip in association with a stomatitis ulcerosa Quantitative determination of the serum immunoglobulins in this connection (at the age of five years) showed that neither IgA nor IgM were present in the serum The IgG content was 5 31 g/l Agar gel and immuno electrophoresis were not performed At the age of seven the child was referred to our hospital because of increasingly copious purulent sputum and suspicion of bronchiectasis The height and weight lay at P90 and P70 for the age respectively Rhinitis mucopurulenta was observed and at auscultation of the lungs diffusely spread rhonchi and crepitations were heard in both lower lobes The teeth were carious The physical examination showed no other anomalies

The sedimentation rate of the erythrocytes was 25 mm The haemoglobin and haematocrit values were normal The blood count showed leucocytosis (21 3×10 /l) the differential count showed 1% eosinophils 2% basophils 4% bandforms 79% segmental neutrophils and 14% lymphocytes Normal serum levels were found for electrolytes calcium inorganic phosphate alkaline phosphatase urea creatinine transaminases cholesterol and uric acid The calcium content of the urine was normal The sweat test gave normal results The blood group was 0 rhesus positive The total haemolytic complement content was elevated

Radiological investigation of the skeleton and bone scintigraphy showed no anomalies X rays of the nasal sinuses showed sinusitis maxillaris and the thorax X ray showed infiltrative changes in both lower lobes At bronchography the right middle lobe bronchus was found to be narrowed with poor filling of the peripheral region The branches of the right lower lobe bronchus showed irregular borders

The lung function investigation showed a slightly reduced vital capacity i e 1550 ml (normal for height 1800 ml) Culture of the nasal secretion and the sputum yielded an abundance of *Haemophilus influenzae*

The faeces showed *Giardia lamblia* which was treated with metronidazol before functional investigation of the gastrointestinal tract Fat excretion was normal Nitrogen excretion was however elevated (1 4 g/24 h normal <1 g/24 h) (14) This could be partially ascribed to a protein loss since in the $Cr^{51}Cl_3$ test the total excretion of the marker over a 4-day period amounted to 10% (normal <1%) D xylose absorption was normal and lactose uptake was reduced The jejunum biopsy specimen showed moderate and in some places even total atrophy of the villi as well as signs of chronic enteritis The lactase level in the biopsy specimen was low (0 8 µmol/g wet weight) The levels of sucrase isomaltase and maltase were normal

IMMUNOLOGICAL INVESTIGATIONS

Humoral immunity

Agar gel electrophoresis of the serum showed a small homogeneous band in the gamma globulin region on immuno electrophoresis with specific antisera this band proved to be IgG1 type λ IgG of other subclasses or type κ was only present in very small amounts The serum IgG content was 5 67 g/l IgM and IgA were not demonstrable and the same holds for isohaemagglutinins antistaphylolysine antistreptolysine and antibodies against the mumps and measles viruses Antibodies against diphtheria tetanus and poliomyelitis were also undemonstrable even after a booster injection of DTP vaccine No immunoglobulin bearing B lymphocytes were present in the peripheral blood or bone marrow and no immunoglobulin containing plasma cells were found in the latter Plasma cells found in the biopsy specimens from the jejunum and rectum contained IgG type λ but plasma cells with IgA or IgM were not present In the nasal secretion IgG of type λ was found but not IgA or IgM The urine did not contain Bence Jones protein

Cellular immunity

The number of lymphocytes in the peripheral blood was always more than 2 0×10⁹/l Delayed type hypersensitivity to mumps was present (induration 15 mm) After sensibilization with dinitrochlorobenzene (DNCB) the challenge led to a positive reaction The percentage of T lymphocytes (E rosettes) was

macytoma with intracellular fibrils Am J Gastroenterol 64 00 1975
11 Hermans P E Diaz Buxo J A & Stobo J D Idiopathic late-onset immunoglobulin deficiency Clinical ob ervations in 50 patients Am J Med 61 1 1876
1 Hewell G M & Alexanian R Multiple myeloma in young persons Ann Intern Med 84 441 1976
13 Hobbs J R Immunocytoma of mice and men Br Med J II 67 1971
14 Kamer J H van de & Wauters E A K Mal absorption Schweiz Med Wschr 106 85 1976
15 Matuchansky C Galian A Goldfain D Rambaud J C Modigliani R & Bernier J J Immunocytes et immunoglobulines du tube digestif au cours des defi its immunitaires primitifs de l adulte Etude de 6 cas Gastr enterol Clin Biol 1 331 1977
16 Mul N A J de Boer L E M Tamineau J A & Ballieux R E Light chain type ratio of IgA producing cells in human jejunal biopsies Eur J Clin Invest 5 63 1975
17 Oxelius V A Monoclonal immunoglobulins in congenital toxoplasmosis Clin Exp Immunol 11 367 197
18 Pena A S Nieuwkoop J Schuyt H R E Hekkens W Th J M & Haex A J Ch Transient paraproteinaemia in a patient with coeliac disease G t 17 735 1976
19 R dl J Dooren L J Morell A Skvaril F Vos en J M J J & Uittenbogaart C H Immunogl bulins and transient paraproteins in sera of patients with the Wiskott Aldrich syndrome a follow up study Cl n Exp Imm in 1 25 256 1976
0 Radl J Dooren L J Eysvoogel V P Went J J van & Hijmans W An immunological study during post tran plantation follow up of a case of severe combined immunodeficiency Clin Exp Im m n l 10 367 197
1 S lomon A Homogeneous (monoclonal) immunogl bul n in cancer Am J Med 63 169 1977
Stoop J W Ballieux R E & Weyers H A Paraproteinaemia with secondary immunoglobulin deficiency in an infant Pediatrics 29 97 196
3 Stoop J W Zegers B J M van der Heiden C & Ballieux R E Monoclonal gammopathy in a child with leukemia Blood 3? 774 1968
24 Stoop J W Eysvoogel V P Zegers B J M Blok Schut Ph D van Bekkum D W & Ballieux R E Selective severe cellular immunodeficiency effect of thymus transplantation and transfer factor administration Clin Immunol Immunopathol 6 289 19 6
25 Vlist G J van der Donckerwolcke R A Kuyten R H & Zegers B J M The haemolytic uraemic syndrome associated with Yersinia enterolitica infection and transient paraproteinaemia (in preparation)
6 Vogler L B Gathings W E Lawton A R & Cooper M D Immunofluorescent studies of the development of pre B cells B lymphocytes and immunoglobulin isotype diversity in humans Eur J Immunol 7 804 1977
27 Vossen J M & Hijmans W Membrane associated determinants on bone marrow and blood lymphocytes in the pediatric age group and on fetal tissues Ann N Y Acad Sci 254 6? 1975
8 Zegers B J M Maertzdorf W J van Loghem E Mul N A J Stoop J W van der Laag J Vossen J M & Ballieux R E Kappa chain deficiency an immunoglobulin disorder N Engl J Med 94 10 6 1976

Submitted May ? 1978
Accepted Aug 9 1978

(B J M Z) Dept of Immunology
University Children s Hospital Het Wilhelmina Kinderziekenhuis
Nieuwe Gracht 137
Utrecht
The Netherland

vided indications suggesting a tumour in the gastrointestinal tract Moreover the morphology of the plasma cells in the intestinal biopsy specimen was normal There are few follow up reports on this kind of patients and relatively little is known about the course although a rapidly fatal development has been documented in a few cases (10) Our patient showed a steady clinical improvement which also makes a malignant plasmacytoma unlikely

The presence of monoclonal IgG in a patient lacking mature B cells is intriguing Recently *the occurrence of precursor B cells* in patients with a congenital agammaglobulinaemia whose bone marrow showed *neither B lymphocytes nor plasma cells* was reported (26) On this basis it is conceivable that in our patient a pre B cell clone might *have escaped the arrest of the pre B cells in congenital agammaglobulinaemia (26) It is* not impossible that intense stimulation at the *level of the gut for instance by bacterial* lipopolysaccharides led to the development of the cell clone found there Broom et al *(3) postulated a similar mechanism to explain* the presence of plasma cells in the jejunum of patients with late onset aggammaglobulinaemia

In our patient antibiotic therapy and gamma globulin injections led to considerable im*provement of the clinical condition A striking* feature was the associated decrease of monoclonal IgG in the serum Although we can only speculate *this observation suggests that the* treatment inhibited the elusive stimulus for synthesis *The decrease of the IgG in the* serum was accompanied by *a decrease in the* density of the plasma cells in the jejunum and an improvement of the villous pattern and the enzyme activity However these last two findings could be purely coincidental because it is highly improbable that successive biopsy specimens *are taken from the same place* (15)

With respect to the prognosis it can be said that humoral immunodeficiency responds well to treatment *The activity of the cell clone in* the intestinal mucosa can be followed rather easily on the basis of quantitative serum IgG determinations performed in serum sampled before successive gammaglobulin injections Whether degeneration of the clone will occur remains to be seen

ACKNOWLEDGEMENTS

We are grateful to Dr J M Vossen Academic Hospital Leiden and Dr N A J Mul Academic Hospital Utrecht who performed the immunofluorescence analysis of bone marrow and jejunum biopsies respectively Dr Erna van Loghem Central Laboratory of the Blood *Transfusion Service Amsterdam* kindly confirmed the subclass determination and light chain typing of the monoclonal immunoglobulin of the patient

REFERENCES

1 Abdou N I Casella S R Abdou N L & *Abrahamsohn I A* Comparative study of bone marrow and blood B cells in infantile and acquired agammaglobulinaemia *J Clin Invest 52* 2218 1973

2 Ament M E Ochs H D & Davis S D Structure *and function of the gastrointestinal tract in primary* immunodeficiency syndromes A study of 39 patients *Medicine 52* 227 1973

3 Broom B C De la Concha E G Webster A D *B* Loeur G & Asherson G L *Dichotomy* between immunoglobulin synthesis by cells in gut and blood of patients with hypogammaglobulinaemia *Lancet II* 253 1975

4 *Cooper M D Lawton A R & Bockman D E* Agammaglobulinaemia with B lymphocytes specific defect of plasma cell differentiation *Lancet II* 791 1971

5 *Danon F & Seligmann M Serum monoclonal* immunoglobulins in childhood *Arch Dis Child* 48 207 1973

6 Eggermont E van & Hers H G The sedimenta*tion properties of the intestinal α glucosidases* of normal human subjects and of patients with sucrose intolerance *Eur J Biochem* 9 488 1969

7 Fernandes J & van de Kamer J H The com*bined xylose disaccharide tolerance test* its application for diagnosing disaccharidase deficiency *Acta Paediatr Scand 60* 187 1971

8 Gatti R A On the classification of patients with primary immunodeficiency disorders *Clin Immunol Immunopathol 3* 243 1974

9 Gmelig Meyling F H J UytdeHaag A G C M & Ballieux R E Human B cell activation in vitro T cell dependent pokeweed mitogen induced differentiation of blood B lymphocytes *Cell Immunol 33* 156 1977

10 Harper T B Powers J M Russell H E Dunn W B & Gardner W A Primary small bowel plas

Acta Pædiatr Scand 68 193–198 1979

A SURVEY OF 164 FINNISH CHILDREN AND ADOLESCENTS WITH HYPERTENSION

M UHARI and O KOSKIMIES

From the Depa tment of Paediatrics Univ ersity of Oulu and Children s Hospital University of Helsinki Finland

ABSTRACT Uhari M and Koskimies O (Department of Paediatrics University of Oulu and Children s Hospital University of Helsinki) A survey of 164 Finnish children and adolescents with hypertension Acta Paediatr Scand 68 193 1979 —A retrospective analysis was performed on 164 children and adolescents with persistent hypertension Among the un selected 115 patients with hypertension seen within the last three years 47 (41%) exhibited renal disease 37 (32%) coarctation of the aorta 10 (9%) miscellaneous associated causes and 21 (18%) no associated cause (essential hypertension) A substantial number 53/164 had a primary disease potentially curable by surgery and in 37 patients the blood pressure was normalized postoperatively The outcome depended mostly on the basic disease and the avail ability of chronic hemodialysis 11/164 children ha e died all because of terminal basic disease and one with simultaneous hypertensive crisis We thus recommend a thorough in vestigation in the case of a child with persistent hypertension

KEY WORDS Children adolescents hypertension blood pressure

In most published series severe hypertension in childhood is found to be mainly secondary in nature most often due to renal diseases or coarctation of the aorta (4 17 20) Gill et al suggest that essential hypertension is rare in children finding only one case in their series of 100 British children with hypertension (4) Little information is available from other paediatric centres in Europe but in the United States the prevalence of essential hyperten sion has been found to be in general higher (1 5 12 15) even up to 95% in one series of mildly hypertensive children (14)

We present here a retrospective analysis of 164 hypertensive children and adolescents examined and followed up in two University Hospitals in Finland The purpose was to find out the main causes of hypertension in child hood and the percentage of children with no detectable primary cause of their hyperten sion

PATIENTS AND METHODS

Patients The study was based on a total of 164 children with hyperten on who had been treated and followed up in the Department of Paediatrics University of Oulu and in the Children s Hospital University of Helsinki 115 of these children had visited the hospitals during the period 1973–1975 and the remaining 49 had been diagnosed and followed up earlier

Criteria for hypertension The following criteria were used for inclusion a diastolic pressure of 90 mmHg or more or a systolic pressure of 130 mmHg or more in children under 5 years or a systolic pressure of 140 mmHg or more in older children all measured repeatedly over 3 months or more These values are well above those published for normal Finnish children (18) The authors are aware that many infants and children with mild or labile hypertension or deficient follow-up are excluded under these criteria (14)

Investigations performed The type of investigation was related to the initial clinical evaluation or diagnosis If necessary one or more of the following examinations were carried out aortography renal angiography cardiac cath eterization renal vein renin samples or renal biopsy Before making the final diagnosis of essential hyperten sion the primary causes of hypertension were excluded by renal function studies urine analyses for bacteria ECG chest roentgenogram intravenous urography hormone estimations and in some cases renal angiography and renin estimations ( )

RESULTS

The age distribution of the 164 patients 63 girls and 101 boys is seen in Fig 1 The mean blood pressure recorded was 163/107 mmHg

Acta Pædiatr Scand 68 193–198 1979

A SURVEY OF 164 FINNISH CHILDREN AND ADOLESCENTS WITH HYPERTENSION

M UHARI and O KOSKIMIES

From the Department of Paediatrics University of Oulu and Children s Hospital University of Helsinki Finland

ABSTRACT Uhari M and Koskimies O (Department of Paediatrics University of Oulu and Children s Hospital University of Helsinki) A survey of 164 Finnish children and adolescents with hypertension Acta Paediatr Scand 68 193 1979 —A retrospective analysis was performed on 164 children and adolescents with persistent hypertension Among the unselected 115 patients with hypertension seen within the last three years 47 (41%) exhibited renal disease 37 (32%) coarctation of the aorta 10 (9%) miscellaneous associated causes and 21 (18%) no associated cause (essential hypertension) A substantial number 53/164 had a primary disease potentially curable by surgery and in 37 patients the blood pressure was normalized postoperatively The outcome depended mostly on the basic disease and the availability of chronic hemodialysis 11/164 children have died all because of terminal basic disease and one with simultaneous hypertensive crisis We thus recommend a thorough investigation in the case of a child with persistent hypertension

KEY WORDS Children adolescents hypertension blood pressure

In most published series severe hypertension in childhood is found to be mainly secondary in nature most often due to renal diseases or coarctation of the aorta (4 17 20) Gill et al suggest that essential hypertension is rare in children finding only one case in their series of 100 British children with hypertension (4) Little information is available from other paediatric centres in Europe but in the United States the prevalence of essential hypertension has been found to be in general higher (1 5 12 15) even up to 95% in one series of mildly hypertensive children (14)

We present here a retrospective analysis of 164 hypertensive children and adolescents examined and followed up in two University Hospitals in Finland The purpose was to find out the main causes of hypertension in childhood and the percentage of children with no detectable primary cause of their hypertension

PATIENTS AND METHODS

Patients The study was based on a total of 164 children with hypertension who had been treated and followed up in the Department of Paediatrics University of Oulu and in the Children s Hospital University of Helsinki 115 of these children had visited the hospitals during the period 1973–1975 and the remaining 49 had been diagnosed and followed up earlier

Criteria for hypertension The following criteria were used for inclusion a diastolic pressure of 90 mmHg or more or a systolic pressure of 130 mmHg or more in children under 5 years or a systolic pressure of 140 mmHg or more in older children all measured repeatedly over 3 month or more These values are well above those published for normal Finnish children (18) The authors are aware that many infants and children with mild or labile hypertension or deficient follow up are excluded under these criteria (14)

Investigations performed The type of investigation was related to the initial clinical evaluation or diagnosis If necessary one or more of the following examinations were carried out aortography renal angiography cardiac catheterization renal vein renin samples or renal biopsy Before making the final diagnosis of essential hypertension the primary causes of hypertension were excluded by renal function studies urine analyse for bacteria ECG chest roentgenogram intravenous urography hormone estimations and in some cases renal angiography and renin estimations ( )

RESULTS

The age distribution of the 164 patients 63 girls and 101 boys is seen in Fig 1 The mean blood pressure recorded was 163/107 mmHg

Acta Pædiatr Scand 68 193–198 1979

A SURVEY OF 164 FINNISH CHILDREN AND ADOLESCENTS WITH HYPERTENSION

M UHARI and O KOSKIMIES

From the Department of Paediatrics Uni ersity of Oulu and Children s Hospital University of Helsinki Finland

ABSTRACT Uhari M and Koskimies O (Department of Paediatrics University of Oulu and Children s Hospital University of Helsinki) A survey of 164 Finnish children and adoles cents with hypertension Acta Paediatr Scand 68 193 1979 —A retrospective analysis was performed on 164 children and adolescents with persistent hypertension Among the un selected 115 patients with hypertension seen within the last three years 47 (41%) exhibited renal disease 37 (32%) coarctation of the aorta 10 (9%) miscellaneous associated causes and 21 (18%) no associated cause (essential hypertension) A substantial number 53/164 had a primary disease potentially curable by surgery and in 37 patients the blood pressure was normalized postoperatively The outcome depended mostly on the basic disease and the avail ability of chronic hemodialysis 11/164 children have died all because of terminal basic dis ease and one with simultaneous hypertensive crisis We thus recommend a thorough in vestigation in the case of a child with persistent hypertension

KEY WORDS Children adolescents hypertension blood pressure

In most publi hed series severe hypertension in childhood is found to be mainly secondary in nature most often due to renal diseases or coarctation of the aorta (4 17 20) Gill et al suggest that essential hypertension is rare in children finding only one case in their series of 100 British children with hypertension (4) Little information is available from other paediatric centres in Europe but in the United States the prevalence of essential hyperten sion has been found to be in general higher (1 5 12 15) even up to 95% *in one series of* mildly hypertensive children (14)

We present here a retrospective analysis of 164 hypertensive children and adolescents examined and followed up in two University Hospitals in Finland The purpose was to find out the main causes of hypertension in child hood and the percentage of children with no detectable primary cause of their hyperten sion

PATIENTS AND METHODS

Patients The study was based on a total of 164 ch ldren with hypertension who had been treated and followed up in the Department of Paediatrics University of Oulu and in the Children s Hospital University of Helsinki 115 of these children had visited the hospitals during the period 1973–1975 and the remaining 49 had been diagnosed and followed up earlier

Criteria for hypertension The following criteria were used for inclusion a diastolic pressure of 90 mmHg or more or a systolic pressure of 130 mmHg or more in children under 5 years or a systolic pressure of 140 mmHg or more in older children all measured *repeatedly over* 3 months or more These values are well above those published for normal Finnish children (18) The authors *are aware that* many infants and children with *mild or* labile hypertension or deficient follow up are excluded under these criteria (14)

Investigations performed The type of investigation was related to the initial clinical evaluation or diagnosis If necessary one or more of the following examina ions were carried out aortography renal angiography cardiac cath eterization renal vein renin samples or renal biopsy *Before making the final* diagnosis of essential hyperten sion the primary causes of hypertension were ex luded by renal function studies urine analyses for bacteria ECG chest roentgenogram intravenous urography hormone e timations and in some cases renal angiography and renin estimations ( )

RESULTS

The age distribution of the 164 patients 63 girls and 101 boys is seen in Fig 1 The mean blood pressure recorded was 163/107 mmHg

Table 2 *Diagnoses associated with hypertension by age groups*

Only patients followed up during the years 1973–1975 are representative for calculating the percentage distribution of the causes of hypertension

Diagnosis	I Patients followed up during 1973–1975 Number of patients by age groups 0–5	5–10	10–16	Total (%)	II Other patients	Total
Renal disease				47 (41%)	36	83
Chronic glomerular disease	1	4	7	12	15	
Pyelonephritis				9	6	
With reflux		4	3			
Without reflux		1	1			
Obstructive uropathy	1	3	3	7	2	
Polycystic kidneys	3	1	1	5	1	
Renovascular disease	1	2	1	4	2	
Dysplastic kidneys	4			4		
Nephronophthisis		1	1	2	2	
Nephropathia		1	1	2	5	
Interstitial nephritis		1		1	3	
Hemolytic uremic syndrome		1		1		
Coarctation of the aorta	13	14	10	37 (32%)	2	39
Arteriosclerosis juvenilis		1	1	2	1	3
Turner's syndrome		1	1	2	1	3
Phaeochromocytoma			1	1	2	3
Nephroblastoma	1			1	1	2
Leukaemia (renal damage)		1		1		1
Pseudohypoparathyreosis			1	1		1
Obesity				2		2
Essential hypertension				21 (18%)	6	27
Obvious	1	2	9	1		
Suspected			7	9		
Total				115	49	164

The total of 164 patients contained 13 in which no reason could be found for the elevated blood pressure, renal arteriography being normal. These patients fulfilled the criteria of essential hypertension (2). A further 14 patients were suspected to have essential hypertension since no obvious primary disease was detected but as renal arteriography had not been performed the diagnosis could not be established with certainty. Eight of these children had labile hypertension with their blood pressure elevated for three months or more but recorded as normal on some occasions afterwards. Essential hypertension occurred in 21 (18%) of the 115 patients. Obesity was the only finding in two children with hypertension.

Table 3 *Outcome in 164 hypertensive patients followed up for 1–17 years (mean 4 years)*

Outcome	No of patients
Cured (no longer receiving treatment)	
By operation	37
Weight loss	1
Under medication	57
Under observation	43
Dead (one after transplantation)	11
Successful renal transplantation	6
Hemodialysis after unsuccessful transplantation	3
Hemodialysis (and medication)	2
Not known	4
Total	164

Treatment and outcome The follow up time varied from one year to 17 years with a mean of 4 years. 59 children received continuous medical treatment and 43 were followed up untreated (Table 3). The latter included 6 with coarctation of the aorta waiting for an operation at the time of compiling the data. Diuretics were the most common antihypertensive preparation used and methyldopa, reserpin, clonidine and hydralazine were also used. The

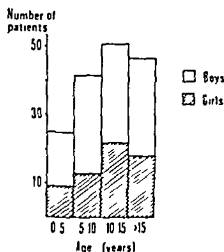


Fig. 1. Distribution of the 164 hypertensive children by sex and age at the time of the survey.

Reasons for referral. 48 of the children had been referred to hospitals because of elevated blood pressure, including 17 who were symptomless and were identified at routine screening in a Child Welfare Centre or in a school health examination. The other children had symptoms including cardiac murmur, proteinuria, urinary tract infection or cardiac insufficiency (Table 1).

Causes of hypertension. The numerical and percentage distributions of the conditions associated with hypertension in the patients followed up during the period 1960–1975 are depicted in Table 2. Since cardiological patients could not be identified accurately from the earlier years, as seen in Table 2, the patients examined during the years 1973–1975 are thus documented separately.

The most common cause of hypertension in these 115 cases was found to be renal disease. Chronic glomerular disease was found in 12 children, four with primary and eight with secondary glomerular disease. The latter ones had the following main diagnoses: three patients had anaphylactoid purpura, three had collagen disease, one had hydrocephalus and shunt nephritis and one had Alport's syndrome. In the category of pyelonephritis seven patients had moderate to severe vesicoureteral reflux (Grades III–V) and two had no reflux at all at the time when hypertension was discovered (6). The diagnosis of pyelonephritis was based on repeated positive urine culture results and pathological findings at intravenous urography, voiding cystography, renal angiography or renal biopsy. One child had interstitial nephritis due to prolonged phenacetin ingestion. The earlier part of the series included one boy with interstitial nephritis due to hypercalcemia from vitamin D overdosage.

One of the patients listed under "nephropathia" had renal biopsy changes that were not clearly classifiable and another had longstanding juvenile diabetes mellitus with renal damage. Renal damage developed in one patient during the induction treatment for leukaemia. One of the two patients with nephroblastoma was first detected due to a hypertensive crisis, while the other developed hypertension at the age of 15, having been in good health for ten years after the treatment of the primary tumour and metastases in the lung with surgery and radiotherapy.

Coarctation of the aorta was the most frequently observed single condition causing hypertension, being present in a total of 37 children (32%). Three patients also had a vascular lesion, severe arteriosclerosis and systolic hypertension. These cases are reported elsewhere (8).

Table 1. *Main clinical symptoms or signs leading to admission of the hypertensive children.*

Symptom or sign	No. of patients
High blood pressure with other symptom	31
Cardiac murmur	21
Proteinuria	18
High blood pressure without other symptom	17
Urinary tract infection	17
Cardiac insufficiency	11
Hematuria	8
Headache	7
Convulsions	6
Anemia	4
Growth retardation	3
Others (palpable tumour, renal insuff. etc.)	21
Total	164

Table 2 *Diagnoses associated with hypertension by age groups*

Only patients followed up during the years 1973–1975 are representative for calculating the percentage distribution of the causes of hypertension

Diagnosis	I Patients followed up during 1973–1975				II Other patients	Total
	Number of patients by age groups					
	0–5	5–10	10–16	Total (%)		
Renal disease				47 (41%)	36	83
Chronic glomerular disease	1	4	7	12	15	
Pyelonephritis				9	6	
With reflux		4	3			
Without reflux		1	1			
Obstructive uropathy	1	3	3	7	2	
Polycystic kidneys	3	1	1	5	1	
Renovascular disease	1	-	1	4	-	
Dysplastic kidneys	4			4		
Nephronophthisis		1	1	2	2	
Nephropathia		1	1	2	5	
Interstitial nephritis		1		1	3	
Hemolytic uremic syndrome		1		1		
Coarctation of the aorta	13	14	10	37 (32%)	2	39
Arteriosclerosis juvenili		1	1	2	1	3
Turner's syndrome		1	1	2	1	3
Phaeochromocytoma			1	1	2	3
Nephroblastoma	1			1	1	2
Leukaemia (renal damage)		1		1		1
Pseudohypoparathyreosis			1	1		1
Obesity		2		2		2
Essential hypertension				21 (18%)	6	27
Obvious	1		9	12		
Suspected			7	9		
Total				115	49	164

The total of 164 patients contained 13 in which no reason could be found for the elevated blood pressure, renal arteriography being normal. These patients fulfilled the criteria of essential hypertension (2). A further 14 patients were suspected to have essential hypertension since no obvious primary disease was detected, but as renal arteriography had not been performed the diagnosis could not be established with certainty. Eight of these children had labile hypertension with their blood pressure elevated for three months or more but recorded as normal on some occasions afterwards. Essential hypertension occurred in 21 (18%) of the 115 patients. Obesity was the only finding in two children with hypertension.

Table 3 *Outcome in 164 hypertensive patients followed up for 1–17 years (mean 4 years)*

Outcome	No of patients
Cured (no longer receiving treatment)	
By operation	37
Weight loss	1
Under medication	57
Under observation	43
Dead (one after transplantation)	11
Successful renal transplantation	6
Haemodialysis after unsuccessful transplantation	3
Haemodialysis (and medication)	
Not known	4
Total	164

Treatment and outcome. The follow up time varied from one year to 17 years, with a mean of 4 years. 59 children received continuous medical treatment and 43 were followed up untreated (Table 3). The latter included 6 with coarctation of the aorta waiting for an operation at the time of compiling the data. Diuretics were the most common antihypertensive preparation used, and methyldopa, reserpin, clonidine and hydralazine were also used. The

Table 4 Types and outcome of surgical intervention in 64 hypertensive children

Diagnosis	Operation	No of patients	BP normalized in
Coarctation of the aorta	Reconstruction	35	26
Unilateral renal disease	Nephrectomy	6	6
Reflux nephropathy	Antireflux operation	5	
Phaeochromocytoma	Extirpation of tumour	3	2
Renovascular disease	Arterial reconstruction	2	-
	Nephrectomy	1	1
Renal insufficiency	Transplantation	10	
Obstructive uropathy	Resection of urethral valve	1	
Shunt nephritis	Removal of the shunt	1	
Total		64	37

medical treatment was effective in all cases of essential hypertension

A total of 64 patients had been operated upon mainly for reconstruction of the aorta (Table 4) Out of the total of 164 children the blood pressure of 38 was completely normalized 37 after surgical treatment and one after losing weight (Table 3) Ten children underwent renal transplantation of which there were four rejected

There were eight children with hypertensive crisis which were successfully treated Nine children died during the follow up because of a renal disease (Table 3) the primary disease being pyelonephritis in three of these cases and glomerular disease in the others Hypertension became worse in these cases as renal insufficiency advanced One child died of nephroblastoma and hypertensive crisis and one of those with severe arteriosclerosis died during the follow up period

DISCUSSION

Hypertension is an important factor when considering morbidity and mortality in adults and it is a cause of disease and even death in childhood and infancy (11 13 17) Childhood hypertension and blood pressure are of interest because of the possibility that they may be markers of future vascular complications (1 22)

It has been claimed that childhood hypertension is not so rare as it has been found to occur with a prevalence of 1 4–8 2% in children and adolescents (3 12 15) Of course hypertension can be defined in quite different ways e g statistically (those individuals exceeding the mean by 2 S D or more) or by empirical determined numerical blood pressure value e g 140/90 mmHg Thus the prevalence quoted are not always comparable (7) Our material was selective from this point of view and no prevalence figure can be calculated

The measurement of blood pressure as routine part of the physical examination does not yet seem to work well in Finland since only approximately every fourth child had his/her blood pressure measured by the primary care physician before referral to hospital Even so 17 children had been referred precisely because their blood pressure was found to be high in routine health screening

The same etiological factors can cause hypertension in children and adults (16 17) but most authors claim that the possibilities of finding a curable cause for the hypertension are clearly greater in children than in adults (4) This has been challenged by some however (14) Our series although somewhat selective clearly showed that in properly investigated patients secondary hypertension was found in more than 80% of all hypertensive children and adolescents

The most common cause of hypertension in younger patients was coarctation of the aorta

(Table 2) The proportion of cases with renal disease was greater among the older children This is understandable as patients with coarctation are cured early whereas most acquired glomerular diseases first develop at school age and need time to progress before the appearance of hypertension

Pyelonephritis was associated with hypertension in 9 (8%) of the 115 patients If hypertension is caused by pyelonephritis and vesicoureteral reflux there should be renal damage present (13) However Schapiro demonstrated experimentally in rats that hypertension can increase the suspectibility to pyelonephritis (19) It is possible that our two cases of pyelonephritis without renal damage first had hypertension and later developed pyelonephritis

Although renal disease and coarctation of the aorta were the main causes of hypertension essential hypertension was identified in 18% of our patients It is probable that the proportion of essential hypertension would be greater in an epidemiological study (1 10 21) This contrasts with the results of Gill et al whose study represented highly selected cardiac and renal patients with an even higher mean blood pressure (4)

The outcome for children with hypertension depends first of all on the basic disease (20) Patients with hypertension due to an incurable disease fare better when the hypertension is controlled using antihypertensive medication and even renal function might improve (9) The attitude of paediatricians to the treatment of hypertension has been conservative which explains our finding that 43 patients with obvious hypertension were placed under observation without treatment All the children who died during the follow up period suffered from severe incurable disease but as in the other cases their hypertension could be kept under control by medication or surgical intervention

Since 53 out of the 164 patients had surgically curable disease coarctation renovascular disease or unilateral renal disease patients with hypertension should be thoroughly investigated Thus we completely disagree with authors who advocate a passive attitude to the examination of children with hypertension (14)

The even more difficult and still unsolved question is how to screen for hypertension in children and particularly in infants The best available screening method would include the routine measurement of blood pressure careful palpation of femoral pulses and urine analysis all of which should be a part of a normal child welfare examination

ACKNOWLEDGEMENT

Financial support to this study was obtained from the Sigrid Juselius Foundation Finland

REFERENCES

1 Aschinberg L C Zeis P M Miller R A John E G & Chan L Essential hypertension in childhood *JAMA 238* 3 ? 1977

Barrat T M Hypertension in childhood *In* D Williams (ed) *Urology in childhood* Encyclopedia of Urology Suppl XV Springer Verlag New York 1974 p 34

3 Cassimos C H Varlamis G Karamperis S & Katsouyannopoulos V Blood pressure in children and adolescents *Acta Paediatr Scand 66* 439 1977

4 Gill D G daCosta B M Cameron J S Joseph M C Ogg C S & Chantler C Analysis of 100 children with severe and persistent hypertension *Arch Dis Child 51* 951 1976

5 Haggerty R J Maroney M W & Nadas A S Essential hypertension in infancy and childhood Differential diagnosis and therapy *Am J Dis Child 9?* 535 1956

6 Heikel P E & Parkkulainen K V Vesico-ureteric reflux in children A classification and results of conservative treatment *Ann Radiol 9* 1 1966

7 Julius S Classification of hypertension *In* J Genest E Koiw & O Kuchel (eds) *Hypertension* McGraw Hill New York 1977 p 9

8 Kaitila I Rapola J Selonen A Lappalainen J & Joki T A new type of severe arteriosclerosis beginning in childhood *Symposium on clinical genetics* Oslo 1977

9 Kincaid Smith P Renal disease and hypertension *Med Clin North Am 61* 611 1977

10 Lieberman E Essential hypertension in children and youth A pediatric perspective *J Pediatr 85* 1 1974

11 Ljungqvist A & Wallgren G Unilateral renal artery stenosis and fatal arterial hypertension in a newborn infant *Acta Paediat Scand 51* 575 196?

1 Loggie J M H Prevalence of hypertension and distribution of causes *In* M I New & L S Levine

(eds) *Juvenile hypertension* Kroc Foundation series vol 8 Raven Press New York 1977 p 1

13 Loggie J M Systemic hypertension in children and adolescents Causes and treatment *Pediatr Clin North Am 18* 1273 1971

14 Londe S Goldring D Gollub S W & Hernandez A Blood pressure and hypertension in children Studies problems and perspectives *In* M I New & L S Levine (eds) *Juvenile hypertension* Kroc Foundation series vol 8 Raven Press New York 1977 p 13

15 Masland R Heald F Goodale W & Gallagher J Hypertensive vascular disease in adolescence *New Engl J Med 255* 894 1956

16 Pickering G *Hypertension Causes consequences and management* Churchill Livingstone Edinburgh 1974

17 Rance C P Arbus G S Balfe J W & Kooh S W Persistent systemic hypertension in infants and children *Pediatr Clin North Am 21* 801 1974

18 Rasanen L Ahlstrom A & Kantero R L Nutrition survey of Finnish rural children *Ann Acad Sci Fennicae A V Medica 168* 1 1975

19 Shapiro A Experimental pyelonephritis and hypertension Implications for the clinical problem *Ann Int Med 59* 37 1963

20 Still J L & Cottom D Severe hypertension in childhood *Arch Dis Child* 49 313 1974

21 Uhari M Unpublished data

22 Zinner S H Martin L F Sacks F Rosner B & Kass E A longitudinal study of blood pressure in childhood *Am J Epid 100* 437 1975

Submitted April 6 1978
Accepted Aug 24 1978

(M U) Department of Paediatrics
University of Oulu
90220 Oulu 22
Finland

FREE AND BOUND TRYPTOPHAN IN HUMAN PLASMA DURING THE PERINATAL PERIOD

M D TRICKLEBANK F J PICKARD and S W DE SOUZA

From the Department of Child Health University of Manchester Manchester England

ABSTRACT Tricklebank M D Pickard F J and De Souza S W (Department of Child Health University of Manchester Manchester England) Free and bound tryptophan in human plasma during the perinatal period Acta Paediatr Scand 68 199 1979.—The concentration of tryptophan and the degree of binding of the amino acid to protein were examined in human plasma during the perinatal period Both total and unbound (free) tryptophan were higher in cord vein plasma than in the maternal circulation the concentration gradient being approximately 1 2 The proportion of the total plasma tryptophan concentration that was not bound to protein was less in cord vein plasma than in the maternal circulation After birth the proportion in infant plasma fell significantly Both total and free tryptophan fell during the first 24 hours of postnatal life Total tryptophan returned to the cord vein plasma level 6–8 days after birth whilst free tryptophan failed to increase during the period of the observations In premature infants total and free tryptophan also declined in concentration 12–24 hours after birth suggesting the phenomenon to be related to birth rather than to gestational age Phenylalanine remained unchanged whilst tyrosine increased in concentration during the first 80 hours of postnatal life Thus the availability of tryptophan to the tissues appears to decline during the immediate postnatal period and the results suggest that the requirement for tryptophan during this time may exceed the supply from standard artifical milk preparations

KEY WORDS Human neonate plasma free bound tryptophan

Tryptophan is unique amongst the plasma amino acids in that in both human and rodent blood between 80% and 90% circulates bound to serum albumin (13) Factors influencing tryptophan albumin binding have received much attention recently since apart from the role of the amino acid in protein synthesis the availability of tryptophan is an important factor in the control of the synthesis of the neurotransmitter 5 hydroxytryptamine in brain (9 12) The binding of tryptophan to albumin is susceptible to disturbance by many factors Thus the intake of food (23 29) stressful manipulation (18) and the administration of certain pharmacological agents (15) may increase the concentration of free tryptophan in plasma increases in brain tryptophan and 5 hydroxytryptamine turnover often occurring concomitantly (7 15 18) Disturbances in the disposition of tryptophan in plasma occur in a number of neuropathological states (3 6 30) and there is some evidence that the subsequent alteration in brain tryptophan metabolism may have functional consequences (34)

A notable exception to the apparent necessity for maintaining this control over plasma tryptophan exists in the neonatal rat Over 90% of the total plasma tryptophan concentration has been found to be in the free form for up to 60 hours after birth (4 5) Thereafter binding to albumin increases such that only about 40% is free around 5 days of age (4 33) The high proportion of tryptophan in the free form in the plasma of newborn animals is associated with both high brain tryptophan concentration and a synthesis rate of 5 hydroxytryptamine close to its maximal value (4)

It is not known however whether similar changes in the disposition of tryptophan occur in human plasma during the perinatal period Studies of the maternal fetal and neonatal

Table 1 *Total and free tryptophan in maternal and cord vein plasma following induced o spontaneous labour*

Mean values ±1 S D of 8 samples are shown *The significance of differences were assessed by one way analys of variance followed by paired comparisons t test*

	Tryptophan (nmol/ml)		% free tryptophan
	Total	Free	
Maternal plasma 1—before induction *of labour*	53.31±8.48	12.18±3.91	22.54±4.99
Maternal plasma 2—induced labour *well established*	48.74±11.55	12.93±4.71	26.83±8.35
Cord plasma	101.47±27.24	20.46±6.27	18.39±5.50
Significance of differences of cord plasma values from maternal samples 1 and 2 *(p)*	<0.001	<0.001	<0.05
Cord plasma—*spontaneous labour*	111.07±25.09	15.68±5.00	14.57±4.83

plasma aminogram have tended to exclude tryptophan often for methodological reasons (19 20 21 37) and as a consequence little is known of the factors that may influence the availability of tryptophan to the tissues in early life This information may be of clinical significance since there is evidence that insults such as perinatal asphyxia and early under nutrition may alter plasma tryptophan and cerebral 5 hydroxytryptamine metabolism (16 33) changes which may be important for subsequent neuronal development In the present study the binding of tryptophan to albumin in human plasma has been examined during the immediate postnatal period and an attempt has been made to determine the extent to which the disposition of tryptophan in plasma may be influenced by events or procedures peculiar to the perinatal period

METHODS

The sample included infants born by normal vertex vaginal delivery following spontaneous labour or labour induced by rupture of membranes and infusion of oxytocin (Syntocinon Sandoz Products Ltd London U K) All babies were breathing spontaneously by 1 min after birth The gestational ages of premature babies ranged from 28 to 36 weeks and that of term babies from 37 to 43 weeks The birth weights were appropriate for gestational age (26) All babies received their first milk feed (SMA Wyeth Laboratories Maidenhead U K) by 3 hours of age 90 ml/kg body weight in the first 24 hours 120 and 150 ml/kg on the second and third days respectively and 180 ml/kg on subsequent days During the period of study all subject were devoid of clinical abnormalities

Blood was collected from mothers by venepun before the onset of and during established labour fro the umbilical cord vein at birth and from babies by he stab (using minimum pressure) at various times after birt Unless stated otherwise only one sample of blood wa obtained from any one infant All samples were obtaine with the informed consent of the mothers

Blood samples were mixed with heparin (approximatel 0 3 mg/ml blood) and stored at 4 C in sealed container for less than 24 hours before centrifugation at 1000 g f 10 min at room temperature Plasma was withdrawn an immediately stored at −20°C After thawing free an bound tryptophan in plasma were separated by ultr filtration as previously described (18) except that sample were centrifuged at 450 g for 10 min at 37 C Tryptopha was determined in plasma (total tryptophan) and in th plasma ultrafiltrate (free tryptophan) by the method o Denckla & Dewey (11) Tyrosine and phenylalanine wer determined in plasma by the methods of Waalkes & Udenfriend (36) and McCaman & Robins (24) respectively

Recovery of known amounts of the amino acids adde to plasma was between 90% and 100% Duplicates dif fered by less than 5% In the study of the effects o postnatal age on plasma amino acid levels each assay contained at least 3 cord plasma samples and a balance representation of samples from the range of postnatal age considered Storage of blood for 24 hours at 4 C prior t the separation of plasma had a negligible effect on th results

RESULTS

Total and free tryptophan in maternal and cord plasma

The total and free concentration of trypto phan in cord plasma did not differ significantly

Table 2 *Total and free tryptophan in plasma from premature infants*

Mean values ±1 S D of 6 samples are shown The significance of differences was assessed by the paired-comparisons test

	Tryptophan (nmol/ml)		% free tryptophan
	Total	Free	
Cord plasma	89 13±11 01	11 55±4 52	12 93±4 77
Plasma from blood taken 12–24 hours after birth	48 34±12 88	6 96±2 78	12 20±4 60
Significance of differences (p)	<0 01	<0 02	N S

N S = not significant

between infants born following spontaneous or induced labour (Table 1) Total and free tryptophan in cord plasma were however 208% and 158% respectively of the concentration in the maternal circulation sampled when induced labour was well established (Table 1) In cord plasma about 18% of the total tryptophan concentration was in the free form significantly less than the percentage in the maternal circulation both before (22%) and after (26%) the onset of labour (percent free tryptophan Table 1) There was no significant difference between the two maternal samples with respect to total tryptophan free tryptophan or the percent free tryptophan (Table 1)

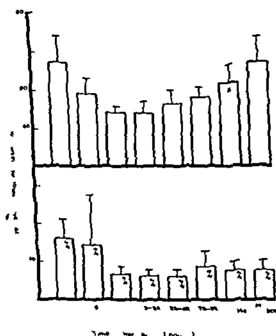


Fig 1 Total and free tryptophan in human cord blood plasma and in heel prick samples taken at various times after birth Bars represent 1 S D Numbers in upper histogram represent the number of samples in each group Percentages refer to the percentages of the plasma total tryptophan concentration that was not bound to protein (percent free tryptophan) The significance of differences were assessed by analysis of variance followed by t test Significantly different from cord vein plasma p<0 001 Significantly different from samples from subjects less than 6 hours old p<0 001

Total and free tryptophan in plasma during the neonatal period

After birth the percentage of free tryptophan declined significantly from 18±4% (mean ±1 S D) to 12±3% by 12 hours after birth (p<0 005 Fig 1) More dramatically both the total and free concentrations of tryptophan fell rapidly after birth Total tryptophan was only 50% of the cord value in samples collected between 7 and 24 hours of postnatal age (Fig 1) Thereafter there appeared to be a gradual recovery and 6–8 days after birth the concentration was not significantly different from that of cord blood During the first 6 hours of life free tryptophan was highly variable but by 7–12 hours after birth it was only 42% of the cord level Unlike total tryptophan free tryptophan remained low throughout the duration of the subsequent observations

Total and free tryptophan in plasma from premature infants

Plasma total and free tryptophan were determined in cord blood from premature infants

Table 1. *Total and free tryptophan in maternal and cord vein plasma following induced or spontaneous labour*

Mean values ±1 S.D. of 8 samples are shown. The significance of differences were assessed by one way analysis of variance followed by paired comparisons *t* test

	Tryptophan (nmol/ml)		% free tryptophan
	Total	Free	
Maternal plasma 1—before induction of labour	53.31±8.48	12.18±3.91	22.54±4.99
Maternal plasma 2—induced labour well established	48.74±11.55	12.93±4.71	26.83±8.35
Cord plasma	101.47±27.24	20.46±6.27	18.39±5.50
Significance of differences of cord plasma values from maternal samples 1 and 2 (*p*)	<0.001	<0.001	<0.05
Cord plasma—spontaneous labour	111.07±25.09	15.68±5.00	14.57±4.83

plasma aminogram have tended to exclude tryptophan often for methodological reasons (19, 20, 21, 37) and as a consequence little is known of the factors that may influence the availability of tryptophan to the tissues in early life. This information may be of clinical significance since there is evidence that insults such as perinatal asphyxia and early under-nutrition may alter plasma tryptophan and cerebral 5-hydroxytryptamine metabolism (16, 33), changes which may be important for subsequent neuronal development. In the present study, the binding of tryptophan to albumin in human plasma has been examined during the immediate postnatal period and an attempt has been made to determine the extent to which the disposition of tryptophan in plasma may be influenced by events or procedures peculiar to the perinatal period.

METHODS

The sample included infants born by normal vertex vaginal delivery following spontaneous labour, or labour induced by rupture of membranes and infusion of oxytocin (Syntocinon, Sandoz Products Ltd., London, U.K.). All babies were breathing spontaneously by 1 min after birth. The gestational ages of premature babies ranged from 28 to 36 weeks, and that of term babies from 37 to 43 weeks. The birth weights were appropriate for gestational age (26). All babies received their first milk feed (SMA, Wyeth Laboratories, Maidenhead, U.K.) by 3 hours of age: 90 ml/kg body weight in the first 24 hours, 120 and 150 ml/kg on the second and third days respectively and 180 ml/kg on subsequent days. During the period of study all subjects were devoid of clinical abnormalities.

Blood was collected from mothers by venepuncture before the onset of, and during, established labour, from the umbilical cord vein at birth, and from babies by heel stab (using minimum pressure) at various times after birth. Unless stated otherwise only one sample of blood was obtained from any one infant. All samples were obtained with the informed consent of the mothers.

Blood samples were mixed with heparin (approximately 0.3 mg/ml blood) and stored at 4°C in sealed containers for less than 24 hours before centrifugation at 1000 *g* for 10 min at room temperature. Plasma was withdrawn and immediately stored at −20°C. After thawing, free and bound tryptophan in plasma were separated by ultrafiltration as previously described (18) except that samples were centrifuged at 450 g for 10 min at 37°C. Tryptophan was determined in plasma (total tryptophan) and in the plasma ultrafiltrate (free tryptophan) by the method of Denckla & Dewey (11). Tyrosine and phenylalanine were determined in plasma by the methods of Waalkes & Udenfriend (36) and McCaman & Robins (34) respectively.

Recovery of known amounts of the amino acids added to plasma was between 90% and 100%. Duplicates differed by less than 5%. In the study of the effects of postnatal age on plasma amino acid levels each assay contained at least 3 cord plasma samples and a balanced representation of samples from the range of postnatal ages considered. Storage of blood for 24 hours at 4°C prior to the separation of plasma had a negligible effect on the results.

RESULTS

Total and free tryptophan in maternal and cord plasma

The total and free concentration of tryptophan in cord plasma did not differ significantly

Table 2 *Total and free tryptophan in plasma from premature infants*
Mean values ±1 S D of 6 samples are shown The significance of differences was assessed by the paired-comparisons *t* test

	Tryptophan (nmol/ml)		% free tryptophan
	Total	Free	
Cord plasma	89 13±11 01	11 55±4 5	1 93±4 77
Plasma from blood taken 1 -24 hours after birth	58 34±1 88	6 96±? 78	1. ?0±4 60
Signif ance of differences (p)	<0 01	<0 0	N S

N S = not significant

between infants born following spontaneous or induced labour (Table 1) Total and free tryptophan in cord plasma were however 208% and 158% respectively of the concentration in the maternal circulation sampled when induced labour was well established (Table 1) In cord plasma about 18% of the total tryptophan concentration was in the free form signifi cantly less than the percentage in the maternal circulation both before (22%) and after (26%) the onset of labour (percent free tryptophan Table 1) There was no significant difference between the two maternal samples with respect to total tryptophan free tryptophan or the percent free tryptophan (Table 1)

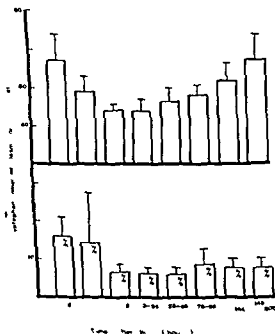


Fig 1 Total and free tryptophan in human cord blood plasma and in heel prick samples taken at various times after birth Bars represent 1 S D Numbers in upper histogram represent the number of sample in each group Percentages refer to the percentages of the plasma total tryptophan concentration that was not bound to protein (percent free tryptophan) The significance of differences were assessed by analysis of variance followed by *t* test
Significantly different from cord vein plasma $p<0.001$
Significantly different from samples from subjects less than 6 hours old $p<0.001$

Total and free tryptophan in plasma during the neonatal period

After birth the percentage of free tryptophan declined significantly from 18±4% (mean ±1 S D) to 12±3% by 12 hours after birth ($p<0.005$ Fig 1) More dramatically both the total and free concentrations of tryptophan fell rapidly after birth Total tryptophan was only 50% of the cord value in samples collected between 7 and 24 hours of postnatal age (Fig 1) Thereafter there appeared to be a gradual recovery and 6–8 days after birth the concentration was not significantly different from that of cord blood During the first 6 hours of life free tryptophan was highly variable but by 7–12 hours after birth it was only 42% of the cord level Unlike total tryptophan free tryptophan remained low throughout the duration of the subsequent observations

Total and free tryptophan in plasma from premature infants

Plasma total and free tryptophan were determined in cord blood from premature infants

Table 1. *Total and free tryptophan in maternal and cord vein plasma following induced or spontaneous labour*

Mean values ±1 S.D. of 8 samples are shown. The significance of differences were assessed by one way analysis of variance followed by paired comparisons *t* test

	Tryptophan (nmol/ml)		
	Total	Free	% free tryptophan
Maternal plasma 1—before induction of labour	53.31±8.48	12.18±3.91	22.54±4.99
Maternal plasma 2—induced labour well established	48.74±11.55	12.93±4.71	26.83±8.35
Cord plasma	101.47±27.24	20.46±6.27	18.39±5.50
Significance of differences of cord plasma values from maternal samples 1 and 2 (*p*)	<0.001	<0.001	<0.05
Cord plasma—spontaneous labour	111.07±25.09	15.68±5.00	14.57±4.83

plasma aminogram have tended to exclude tryptophan often for methodological reasons (19, 20, 21, 37) and as a consequence little is known of the factors that may influence the availability of tryptophan to the tissues in early life. This information may be of clinical significance since there is evidence that insults such as perinatal asphyxia and early under nutrition may alter plasma tryptophan and cerebral 5-hydroxytryptamine metabolism (16, 33), changes which may be important for subsequent neuronal development. In the present study, the binding of tryptophan to albumin in human plasma has been examined during the immediate postnatal period and an attempt has been made to determine the extent to which the disposition of tryptophan in plasma may be influenced by events or procedures peculiar to the perinatal period.

METHODS

The sample included infants born by normal vertex vaginal delivery following spontaneous labour, or labour induced by rupture of membranes and infusion of oxytocin (Syntocinon, Sandoz Products Ltd., London, U.K.). All babies were breathing spontaneously by 1 min after birth. The gestational ages of premature babies ranged from 28 to 36 weeks, and that of term babies from 37 to 43 weeks. The birth weights were appropriate for gestational age (26). All babies received their first milk feed (SMA, Wyeth Laboratories, Maidenhead, U.K.) by 3 hours of age: 90 ml/kg body weight in the first 24 hours, 120 and 150 ml/kg on the second and third days respectively and 180 ml/kg on subsequent days. During the period of study all subjects were devoid of clinical abnormalities.

Blood was collected from mothers by venepuncture before the onset of, and during established labour, from the umbilical cord vein at birth, and from babies by heel stab (using minimum pressure) at various times after birth. Unless stated otherwise only one sample of blood was obtained from any one infant. All samples were obtained with the informed consent of the mothers.

Blood samples were mixed with heparin (approximately 0.3 mg/ml blood) and stored at 4°C in sealed containers for less than 24 hours before centrifugation at 1000 g for 10 min at room temperature. Plasma was withdrawn and immediately stored at −20°C. After thawing, free and bound tryptophan in plasma were separated by ultrafiltration as previously described (18) except that samples were centrifuged at 450 g for 10 min at 37°C. Tryptophan was determined in plasma (total tryptophan) and in the plasma ultrafiltrate (free tryptophan) by the method of Denckla & Dewey (11). Tyrosine and phenylalanine were determined in plasma by the methods of Waalkes & Udenfriend (36) and McCaman & Robins (24) respectively.

Recovery of known amounts of the amino acids added to plasma was between 90% and 100%. Duplicates differed by less than 5%. In the study of the effects of postnatal age on plasma amino acid levels each assay contained at least 3 cord plasma samples and a balanced representation of samples from the range of postnatal ages considered. Storage of blood for 24 hours at 4°C prior to the separation of plasma had a negligible effect on the results.

RESULTS

Total and free tryptophan in maternal and cord plasma

The total and free concentration of tryptophan in cord plasma did not differ significantly

(32) The decline in both total and free tryptophan concentration after birth could reflect the sudden removal of the placental supply route without concomitant adjustment of the uptake and utilisation of the amino acid by the tissues However a similar decline in plasma tryptophan concentration occurs in the rat although in this species the decline does not commence until the animal is at least 30 hours old (4) An alternative explanation that of induction of metabolism along the pyrrolase pathway is unlikely since the concentration of kynurenine and its metabolites is very low in neonatal urine even after ingestion of a tryptophan load (1 38) The phenomenon is at least related to birth rather than to developmental age since a significant fall of both total and free tryptophan was found to occur in premature infants As tryptophan is an essential amino acid it is possible that artificial milk feed preparations (given within 3 hours of birth) are deficient in tryptophan In this respect it is of interest that plasma phenylalanine levels remained essentially unchanged whilst tyrosine steadily increased Feeding experimental animals a diet deficient in tryptophan rapidly depletes both the plasma and the brain of the amino acid (2) and induces a number of behavioural abnormalities probably mediated through decreased formation of 5 hydroxytryptamine (14 22) The present results therefore suggest that more attention should be given to the investigation of the supply and utilisation of tryptophan by the human neonate

ACKNOWLEDGEMENTS

The authors thank the M R C for financial support and Professor J Dobbing in whose laboratories the work was done

REFERENCES

1 Auricchio S Quagliariell E & Rubino A On the presence of metabolites of the tryptophan-nicotinic acid chain in the urine of sucklings during the first month of life II I Paediat Acta 15 479 1960
2 Biggio G Fadda F Fanni P Tagliamonte A & Gessa G L Rapid depletion of serum tryptophan brain tryptophan serotonin and 5 hydroxyindole acetic acid by a tryptophan free diet *Life Sci 14* 13 1 1974
3 Bloxam D L & Curzon G A study of the proposed determinants of brain tryptophan concentration after portacaval anastomosis or sham-operation *J Neurochem* (in press)
4 Bourgoin S Faivre Bauman A Benda P Glowinski J & Hamon M Plasma tryptophan and 5 HT metabolism in the CNS of the newborn rat *J Neurochem 23* 319 1974
5 Bourgoin S Faivre Bauman A Héry F Ternaux J P & Hamon M Characteristics of tryptophan binding in the serum of the newborn rat *Biol Neonate 31* 141 1977
6 Coppen A Eccleston E G & Peet M Plasma tryptophan binding in depression *Adv Biochem Psychopharmac 11* 325 1974
7 Curzon G & Fernando J C R Effect of aminophylline on tryptophan and other aromatic amino acids in plasma brain and other tissues and on brain 5 hydroxytryptamine metabolism *Br J Pharmacol 58* 533 1976
8 Curzon G Friedel J & Knott P J The effect of fatty acids on the binding of tryptophan to plasma protein *Nature 242* 198 1973
9 Curzon G Joseph M H & Knott P J Effects of immobilisation and food deprivation on rat brain tryptophan metabolism *J Neurochem 19* 1967 1972
10 Curzon G & Knott P J Effects on plasma and brain tryptophan in the rat of drugs and hormones that influence the concentration of unesterified fatty acid in the plasma *B J Pharmacol 50* 197 1974
11 Denckla W D & Dewey H K The determination of tryptophan in plasma liver and urine *J Lab Clin Med* 69 160 1967
12 Fernstrom J D & Wurtman R J Brain serotonin content physiological dependance on plasma tryptophan levels *Science 173* 149 1971
13 Fuller R W & Roush B W Binding of tryptophan to plasma proteins in several species *Comp Biochem Physiol 46B* 273 1973
14 Gallup G G Jr Wallnau L B Boren J L Gagliardi G J Maser J D & Edson P H Tryptophan and tonic immobility in chickens effects of dietary and systemic manipulations *J Comp Physiol Psychol 91* 642 1977
15 Gessa G L & Tagliamonte A Possible role of free serum tryptophan in the control of brain tryptophan level and serotonin synthesis *Adv Biochem Psychopharmac 11* 119 1974
16 Kalyansundaram S Effect of dietary protein and calorie deficiency on tryptophan levels in the developing rat brain *J Neurochem 27* 1245 1976
17 Keele D K Kay J L Brown B S & Nordquist B Plasma FFA and blood sugar in mothers and their newborn infants *Pediatrics 37* 597 1966
18 Knott P J & Curzon G Free tryptophan in plasma and brain tryptophan metabolism *Nature 239* 452 1972
19 Lindblad B S The venous plasma free amino acid levels during the first hours of life I After normal and

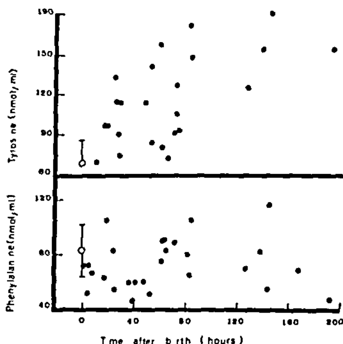


Fig 2 Tyrosine and phenylalanine in infant blood plasma at various times after birth Values at time 0 were obtained from cord vein blood and the accompanying bars indicate 1 S D about the mean value obtained There were 13 and 25 cord vein plasma samples assayed for tyrosine and phenylalanine respectively

and in the same infants 12–24 hours of age were 66% and 60% of the cord plasma values respectively Unlike the immediate postnatal changes seen in full term infants the decline in plasma tryptophan was not accompanied by a significant alteration in the percentage of tryptophan not bound to protein (Table 2)

Tyrosine and phenylalanine in plasma during the neonatal period

No consistent pattern of change in concentration of phenylalanine was found to occur in plasma during the neonatal period whilst plasma tyrosine levels steadily increased (Fig 2)

DISCUSSION

It is well known that the total concentration of α amino nitrogen is higher in cord blood than in the maternal circulation Individual amino acids vary in their concentration gradients across the placenta from about 1 2 to 1 4 there being little in this respect to differentiate the essential from the non essential acids (20 37) Tryptophan does not appear to be an exception to this rule the concentration gradient of both total and free forms being about 1 ?

Significantly less tryptophan was found to be free in cord blood than in the maternal c culation Furthermore the proportion i creased during the first 12 hours of postna life after which only about 12% was free . though this is probably an under estim because the separation did not take place physiological pH and the loss of Co from t sample during preparation and storage favo a rise in pH and the enhancement of bind (25) the results are nevertheless in mark contrast with the newborn rat in which follo ing similar separation procedures more th 80% of the total plasma tryptophan is t bound (4 5)

It has been suggested that the lack of bi ing of tryptophan to albumin in the rat m be due at least in part to high levels of c culating non-esterified fatty acids (NEFA during the neonatal period (4 5) NEFA s co pete with tryptophan for binding to albun (8) and increased lipolysis is associated w increased free tryptophan levels (10 23) T high degree of binding in human cord plas and the subsequent enhancement follow birth may therefore be anomalous since plas NEFA s are reported to rise markedly dur the first 24 hours of postnatal life (17 27 : 35)

A further anomaly perhaps exists in t maternal circulation there was no significa difference in the concentration of total or fr tryptophan or in the percentage free tryp phan between samples taken immediately b fore the onset of labour and when labour w well established NEFA s again probably n markedly during this time (31) Hence t binding of tryptophan would be expected decrease (8 18) This finding may be of intere in view of the recent evidence suggesting a association between disturbances in trypt phan albumin binding and the onset of depre sive symptoms in women following childbir

(32) The decline in both total and free tryptophan concentration after birth could reflect the sudden removal of the placental supply route without concomitant adjustment of the uptake and utilisation of the amino acid by the tissues However a similar decline in plasma tryptophan concentration occurs in the rat although in this species the decline does not commence until the animal is at least 30 hours old (4) An alternative explanation that of induction of metabolism along the pyrrolase pathway is unlikely since the concentration of kynurenine and its metabolites is very low in neonatal urine even after ingestion of a tryptophan load (1 38) The phenomenon is at least related to birth rather than to developmental age since a significant fall of both total and free tryptophan was found to occur in premature infants As tryptophan is an essential amino acid it is possible that artificial milk feed preparations (given within 3 hours of birth) are deficient in tryptophan In this respect it is of interest that plasma phenylalanine levels remained essentially unchanged whilst tyrosine steadily increased Feeding experimental animals a diet deficient in tryptophan rapidly depletes both the plasma and the brain of the amino acid (2) and induces a number of behavioural abnormalities probably mediated through decreased formation of 5 hydroxytryptamine (14 22) The present results therefore suggest that more attention should be given to the investigation of the supply and utilisation of tryptophan by the human neonate

ACKNOWLEDGEMENTS

The authors thank the M R C for financial support and Professor J Dobbing in whose laboratories the work was done

REFERENCES

1 Auricchio S Quagliariello E & Rubino A On the presence of metabolites of the tryptophan nicotinic acid chain in the urine of sucklings during the first month of life *Hel Paediat Acta 15* 479 1960

2 Biggio G Fadda F Fanni P Tagliamonte A & G— L Rapid depletion of serum tryptophan brain tryptophan serotonin and 5 hydroxyindole acetic acid by a tryptophan free diet *Life Sci 14* 1321 1974

3 Bloxam D L & Curzon G A study of the proposed determinants of brain tryptophan concentration after portacaval anastomosis or sham-operation *J Neurochem* (in press)

4 Bourgoin S Faivre Bauman A Benda P Glowinski J & Hamon M Plasma tryptophan and 5 HT metabolism in the CNS of the newborn rat *J Neurochem 23* 319 1974

5 Bourgoin S Faivre Bauman A Héry F Ternaux J P & Hamon M Characteristics of tryptophan binding in the serum of the newborn rat *Biol Neonate 31* 141 1977

6 Coppen A Eccleston E G & Peet M Plasma tryptophan binding in depression *Ad Biochem Psychopharmac 11* 325 1974

7 Curzon G & Fernando J C R Effect of aminophylline on tryptophan and other aromatic amino acids in plasma brain and other tissues and on brain 5 hydroxytryptamine metabolism *Br J Pharmacol 58* 533 1976

8 Curzon G Friedel J & Knott P J The effect of fatty acids on the binding of tryptophan to plasma protein *Nature 242* 198 1973

9 Curzon G Joseph M H & Knott P J Effects of immobilisation and food deprivation on rat brain tryptophan metabolism *J Neurochem 19* 1967 1972

10 Curzon G & Knott P J Effects on plasma and brain tryptophan in the rat of drugs and hormones that influence the concentration of unesterified fatty acid in the plasma *B J Pharmacol 50* 197 1974

11 Denckla W D & Dewey H K The determination of tryptophan in plasma liver and urine *J Lab Clin Med 69* 160 1967

12 Fernstrom J D & Wurtman R J Brain serotonin content physiological dependance on plasma tryptophan levels *Science 173* 149 1971

13 Fuller R W & Roush B W Binding of tryptophan to plasma proteins in several species *Comp Biochem Physiol 46 B* 273 1973

14 Gallup G G Jr Wallnau L B Boren J L Gagliardi G J Maser J D & Edson P H Tryptophan and tonic immobility in chickens effects of dietary and systemic manipulations *J Comp Physiol Psychol 91* 642 1977

15 Gessa G L & Tagliamonte A Possible role of free serum tryptophan in the control of brain tryptophan level and serotonin synthesis *Adv Biochem Psychopharmac 11* 119 1974

16 Kalyansundaram S Effect of dietary protein and calorie deficiency on tryptophan levels in the developing rat brain *J Neurochem 27* 1245 1976

17 Keele D K Kay J L Brown B S & Nordquist B Plasma FFA and blood sugar in mothers and their newborn infants *Pediatrics 37* 597 1966

18 Knott P J & Curzon G Free tryptophan in plasma and brain tryptophan metabolism *Nature 239* 452 1972

19 Lindblad B S The venous plasma free amino acid levels during the first hours of life 1 After normal and

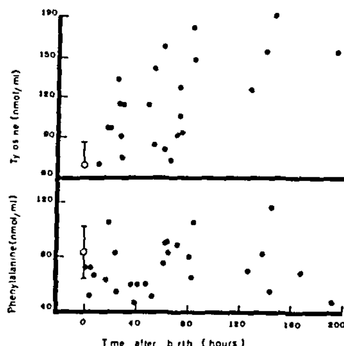


Fig 2 Tyrosine and phenylalanine in infant blood plasma at various times after birth Values at time 0 were obtained from cord vein blood and the accompanying bars indicate 1 S D about the mean value obtained There were 13 and 25 cord vein plasma samples assayed for tyrosine and phenylalanine respectively

and in the same infants 12–24 hours of age were 66% and 60% of the cord plasma values respectively Unlike the immediate postnatal changes seen in full term infants the decline in plasma tryptophan was not accompanied by a significant alteration in the percentage of tryptophan not bound to protein (Table 2)

Tyrosine and phenylalanine in plasma during the neonatal period

No consistent pattern of change in concentration of phenylalanine was found to occur in plasma during the neonatal period whilst plasma tyrosine levels steadily increased (Fig 2)

DISCUSSION

It is well known that the total concentration of α amino nitrogen is higher in cord blood than in the maternal circulation Individual amino acids vary in their concentration gradients across the placenta from about 1 2 to 1 4 there being little in this respect to differentiate the essential from the non essential acids (20 37) Tryptophan does not appear to be an exception to this rule the concentration gradient of both total and free forms being about 1 2

Significantly less tryptophan was found to be free in cord blood than in the maternal circulation Furthermore the proportion decreased during the first 12 hours of postnatal life after which only about 12% was free Although this is probably an underestimate because the separation did not take place at physiological pH and the loss of Co from the sample during preparation and storage favour a rise in pH and the enhancement of binding (25) the results are nevertheless in marked contrast with the newborn rat in which following similar separation procedures more than 80% of the total plasma tryptophan is unbound (4 5)

It has been suggested that the lack of binding of tryptophan to albumin in the rat may be due at least in part to high levels of circulating non-esterified fatty acids (NEFA s during the neonatal period (4 5) NEFA s compete with tryptophan for binding to albumin (8) and increased lipolysis is associated with increased free tryptophan levels (10 23) The high degree of binding in human cord plasma and the subsequent enhancement following birth may therefore be anomalous since plasma NEFA s are reported to rise markedly during the first 24 hours of postnatal life (17 27 28 35)

A further anomaly perhaps exists in the maternal circulation there was no significant difference in the concentration of total or free tryptophan or in the percentage free tryptophan between samples taken immediately before the onset of labour and when labour was well established NEFA s again probably rise markedly during this time (31) Hence the binding of tryptophan would be expected to decrease (8 18) This finding may be of interest in view of the recent evidence suggesting an association between disturbances in tryptophan albumin binding and the onset of depressive symptoms in women following childbirth

Acta Pædiatr Scand 68 205–211 1979

17 HYDROXYPROGESTERONE IN NORMAL CHILDREN AND CONGENITAL ADRENAL HYPERPLASIA

Measurement in Serum by Radioimmunoassay after Thin layer Chromatography

KNUD F PETERSEN and THORKIL CHRISTENSEN

From the Children s Hospital Fuglebakken University of Copenhagen and Medi Lab Copenhagen Denmark

ABSTRACT Petersen K E and Christensen T (The Children s Hospital Fuglebakken University of Copenhagen and Medi Lab Copenhagen Denmark) 17 hydroxyprogesterone in normal children and congenital adrenal hyperplasia Acta Paediatr Scand 68 205 1979— Serum 17α hydroxyprogesterone (17 OH P) was measured by a specific radioimmunoassay technique combined with thin layer chromatography Normal values for children are <1 1 μg/l (<3 3 nmol/l)—corresponding to values found in the literature In congenital adrenal hyperplasia (CAH) values up to several hundred μg/l are found The values rise after ACTH stimulation and are suppressed by decadrone or cortisone treatment The rise in 17 ketosteroids and pregnanetriol in untreated CAH is relatively smaller (15–25 fold) This clinical sensitivity of 17 OH P is thus valuable for the diagnosis of CAH (21 *hydroxylase* deficiency) Furthermore it is easier to take a blood sample than to collect urine for 24 hours The usefulness in therapeutic monitoring is being studied

KEY WORDS 17α hydroxyprogesterone radioimmunoassay thin layer chromatography *children* congenital adrenal hyperplasia

Measurement of serum 17α hydroxyprogesterone (17 OH P) is of interest in congenital adrenal hyperplasia (CAH) Defective 21 hydroxylation is found in the majority (up to 95%) of the patients with the syndrome

17 OH P accumulates and the blood concentration will rise several hundred fold There is a (relatively smaller) rise in the urinary excretion of pregnanetriol Cortisol in plasma is lowered and the negative feed back mechanism leads to further ACTH stimulation of the early steps in the biosynthetic pathway The increased production of androgens will result in a higher excretion of 17 ketosteroids (17 KS) in the urine Diagnosis and therapeutic monitoring of cortisone treatment in CAH rests on the clinical parameters height growth acceleration virilization and bone age development—until recently supplemented by examination of the excretion of total 17 KS and pregnanetriol in the urine In the search for a more sensitive indicator for diagnosis and treatment control and to try to avoid the collection of urine we were interested in developing and evaluation the use of serum 17 OHP

METHODS

17 hydroxyprogesterone method

Reagents Buffer 0 01 M sodium phosphate 0 15 M sodium chloride 0 1% (w/v) sodium azide 0 1 (w/v) gelatine 0 05% (w/v) bovine gamma globulin pH 7 2

1 ? H 17α hydroxyprogesterone 40–60 Ci/mmole (New England Nuclear) was purified by thin layer chromatography prior to use Non radioactive steroids were either obtained from Ikapharm Israel or Steraloids Pawling N Y Dextran-coated charcoal was prepared by mixing 10 g of activated charcoal (Sigma) and 1 g of dextran T 70 (Pharmacia) in 100 ml of buffer Kieselgel G (Type 60) for thin layer chromatography from E Merck was used All other reagents were analytical grade from E Merck

Preparation of antigen 17α hydroxyprogesterone 3 carboxy methoxim was obtained from Steraloids Pawling N Y and coupled to bovine serum albumin (BSA) by the mixed anhydride technique as described by Erlanger et al

short gestation and gestation complicated by hypertension with special reference to the small for-dates syndrome Acta Paediatr Scand 59 13 1970
20 Lindblad B S & Baldesten A The normal venous plasma free amino acid levels of non pregnant women and of mother and child during delivery Acta Paediatr Scand 56 37 1967
21 Lindblad B S & Baldesten A Time studies on free amino acid levels of venous plasma during the neonatal period Acta Paediatr Scand 58 252 1969
22 Lytle L D Messing R B Fisher L & Phebus L Effects of long term corn consumption on brain serotonin and the response to electric shock Science 190 692 1975
23 Madras B K Cohen E L Messing R Munro H N & Wurtman R J Relevance of free tryptophan in serum to tissue tryptophan concentrations Metabolism 23 1107 1974
24 McCaman M W & Robins E Fluorometric method for the determination of phenylalanine in serum J Lab Clin Med 59 885 1962
25 McMenamy R H & Oncley J L The specific binding of L tryptophan to serum albumin J Biol Chem 233 1436 1958
26 Milner R D G & Richards B An analysis of birth weight by gestational age of infants born in England and Wales 1967–1971 J Obstet Gynaecol Br Commonw 81 956 1974
27 Persson B & Gentz J The pattern of blood lipids glycerol and ketone bodies during the neonatal period infancy and childhood Acta Paediatr Scand 55 353 1966
28 Persson B & Tunnell R Influence of environmental temperature and acidosis on lipid mobilisation in the human infant during the first two hours after birth Acta Paediatr Scand 60 385 1971
29 Perez Cruet J Chase T N & Murphy D L Dietary regulation of brain tryptophan metabolism by plasma ratio of free tryptophan and neutral amino acids in humans Nature 248 693 1974
30 Phillipson O T & Bird E D Plasma glucose non esterified fatty acids and amino acids in Huntington's Chorea Clin Sci Molec Med 52 311 1977
31 Sabata U Wolf H & Lausmann S The role of free fatty acids glycerol ketone bodies and glucose in the energy metabolism in the mother and fetus during delivery Biol Neonate 13 7 1968
32 Stein G Milton F Bebbington P Wood K & Coppen A Relationship between mood disturbances and free and total plasma tryptophan in postpartum women Br Med J II 457 1977
33 Tricklebank M D Pickard F J & Dobbing J Tryptophan and 5 hydroxytryptamine metabolism in the immature rat brain during recovery from a physical J Neurochem 28 641 1977
34 Tricklebank M D Smart J L Bloxam D L & Curzon G Effects of chronic experimental liver dysfunction and L tryptophan on behaviour Pharmac Biochem Behav (in press)
35 Van Duyne C M & Havel R J Plasma unesterified fatty acid concentration in fetal and neonatal life Proc Soc Exp Biol Med 102 599 1959
36 Waalkes T P & Udenfriend S A fluorometric method for the estimation of tyrosine in plasma and tissues J Lab Clin Med 50 733 1957
37 Young M & Prenton M A Maternal and fetal plasma amino acid concentrations during gestation and in retarded fetal growth J Obstet Gynaecol Br Comm 76 333 1969
38 Ziegler F & Berger H Tryptophan metabolism in newborn low birthweight infants Clin Chim Acta 28 369 1970

Submitted May 23 1978
Accepted Sept 8 1978

(M D T) Department of Neurochemistry
Institute of Neurology
33 John s Mews
London WC1N 2NS
UK

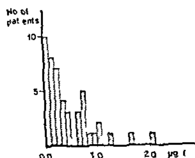


Fig 1 Serum 17α hydroxyprogesterone levels in 47 children

(<7 6 nmol/l)—found in 62 men and 60 women The distribution in women is skewed owing to the midcyclus and luteal phase peak

Normal values for children The values observed in 47 children are shown in Fig 1 The chosen reference interval is <1 1 µg/l (<3 3 nmol/l)—in the first 6 months values up to 2 0 µg/l (6 nmol/l) have been seen

Values in CAH

The results of the 6 patients mentioned here are shown in Table 2 Details and comments are given here

1 At birth this girl was taken to be a boy with hypospadias and testicular retention Sodium crisis at age 20 days (serum sodium 119 mEq/l) At the age of one month the condition was thought to be inadequately suppressed so the cortisone dose was raised from 20 to 30 mg daily As shown this fully suppressed the pathological values

2 *Pseudohermaphroditism at birth and salt loss* Difficult to treat did not take the cortisone properly had long lasting menostasia and was adipose The values are suppressed towards normal values during raised cortisone dose and proper intake

3 This boy received increased cortisone treatment with the effect shown He had also extremely large diurnal variations 11 30 a m 112 11 30 p m 3 6 and 8 00 a m 161 µg/l

4 Pseudohermaphroditism without salt loss Unmanageable for some time with menostasia *and hirsutism took 25 mg cortisone* daily An increase to 50 mg daily did not change the values much but almost complete suppression was achieved with 100 mg daily

5 This girl had an older sister with saltlosing CAH but she had developed quite normally and had never shown any signs of CAH before the age of 5½ when she developed pubic hair The urinary excretion of 17 KS and pregnanetriol was increased and increased

Table 2 *Values for 17 OH P in patients with CAH*

No	Sex	Age studied	Treatment cortisone (mg/24 h)	17 KS (mg/ 4 h)	Pregnanetriol (mg/24 h)	17 OH P (µg/l)
1	f	1/12 y	(*a*) 20	2 3	0 6	184
			(*b*) 30	0 1	0	1 9
2	f	1? y	(*a*) 1 5?	6 8	15 4	99
			(*b*) 137 5?	6 6	7 2	47
			(*c*) 150?	5 5	? 5	1? 1
3	m	8 y	(*a*) 25	? 2	4 9	65
			(*b*) 30	2 0	0 6	1 3
4	f	?4 y	(*a*) 25	31	66	119
			(*b*) 100	3 0	0 6	22
5	f	5½ y	(*a*) 0	2	4 3	8 5
			(*b*) 0+ACTH	8 6	5 9	99
			(*c*) 15	1 7	0 6	1 3
6	f	7 y	(*a*) 0	1 8	1 2	2 0
			(*b*) 0+ACTH	? 9	1 5	0 8

(8) The reaction mixture was dialyzed three times for 24 hours against 100 volumes of 0.15 M sodium chloride. By U.V. spectroscopy the number of steroid residues per mole of BSA was determined to be 11.

Preparation of antisera. The antigen (4 mg/ml in 0.15 M sodium chloride) was mixed with an equal volume of complete Freund adjuvant. Each of six rabbits received subcutaneously 0.1 ml of the suspension once every two weeks for two months. Boosters were then given monthly and blood was drawn 10–12 days after each booster injection.

Assay procedure. Serum was equilibrated with a small amount of tritium labelled 17 OH P denaturated with methanol and extracted with chloroform. After evaporation under nitrogen the extract was purified by thin layer chromatography on silica gel plates with *n* hexane/ethyl acetate/1 2-dichloroethane/water 100/80/20/1 (vol.) as mobile phase. After chromatography elution from the gel and evaporation of solvent the extracts were dissolved in phosphate buffer. An aliquot was counted to measure procedural loss while 17 OH P was quantitated in the remaining part by radioimmunoassay with charcoal separation. All results were corrected individually for procedural losses.

Evaluation

Specificity. The most suitable antiserum was chosen according to the equilibrium constant for the antigen antibody reaction and the cross reactions with other steroids. The equilibrium constant K was 5×10^{10} l/mol calculated from a Scatchard plot.

Measured cross reactions were as shown in Table 1. It will be seen that the accumulated interference was about 20% at physiological concentrations mainly owing to progesterone and cortisol. Analysis of 15 normal sera with and without chromatography confirmed this: the values were (mean) 23% higher without chromatography. In a patient with Cushing's syndrome the value without chromatography was 200% higher. As progesterone rises considerably in women in the luteal phase we found it necessary to add a chromatographic purification step. By thin layer chromatography in the system used 17 OH P was separated from the other steroids mentioned in Table 1. It is to be noted that R_f for cortisol 17 OH P and progesterone is 0.04, 0.39 and 0.61 respectively.

Recovery of 17 OH P added to serum was found to be 104.0±6.5% (S.D.) ($n=22$) corrected for procedural losses. The total loss during procedure was 50±10% (S.D.). Six sera with raised 17 OH P concentration were analyzed at full, half and fifth volume—results were not dependent on the serum volume used.

Reproducibility was studied by analyzing five pools in 12–14 series. The mean coefficient of variation was 6% within the series (sA minimum 0.1 µg/l) and 7% between the series (sA minimum 0.2 µg/l). sA=analytical standard deviation.

Sensitivity. The detection limit defined as two analytical standard deviations is 0.4 µg/l.

Other methods

Total 17 KS in urine. Callow's modification of the Zimmerman reaction (Statens Seruminstitut). Normal values depend on age: the maximum values are given for the different age groups: 0 year 1 mg/24 hrs—5 years 2 mg/24 hrs—10 years 4 mg/24 hrs—15 years 8 mg/24 hrs—20 years 16 mg/24 hrs (women).

Pregnanetriol in urine. Gaschromatography after converting to etiocholanolone (Medi. Lab.). Normal values in children below 1 mg/24 hrs.

Table 1. *Cross reactions with different steroids*

Steroid	Cross reaction	Relative % interference[a]
17α OH Progesterone	≡100	≡100
Progesterone	20	10[b]
17α OH Pregnenolone	3	3
Pregnenolone	0.2	<1
Cortisol	0.02	<6
11 Deoxycortisol	0.6	<3
Corticosterone	0.02	<1
11 Deoxycorticosterone	0.2	<1
Testosterone	0.02	<0.02
5α Dihydrotestosterone	0.02	<0.01
Androstendione	0.02	<0.01
Estradiol 17β	0.002	<0.01

[a] Relative interference signifies cross reaction corrected for the relative physiological serum concentrations.
[b] In women in the luteal phase up to 400%.

MATERIAL

Normal children

From children who had a venipuncture done during the study of their disease 2 ml of serum was taken for analysis. None of the children had clinical or anamnestic signs of hormonal disease (but may have epilepsy, asthma, different infections). The sixteen girls and thirty-one boys were distributed according to age: 8 days to 1 year 13, 1 to 6 years 17, 6 to 15 years 17. The greater part of the blood sampling was done between 8 a.m. and 10 a.m.—a few samples were taken between 11 a.m. and 3 p.m. in the out patient department.

Patients with CAH

The diagnosis was made in the neonatal period in girls with virilized external genitalia (with or without salt loss) and in boys with salt loss—or later when precocious pseudopuberty occurred. The diagnosis rests at increased 17 KS and pregnanetriol excretion which are suppressible with cortisone treatment. In some cases a thorough metabolite pattern excretion was available. All the patients were suffering from 21 hydroxylase deficiency.

RESULTS

Reference values

Reference values for adults (interval between 2.5% and 97.5% fractiles) were <2.5 µg/l

matography the authors conclude that chromatography must be used in all 17 OH P analysis

In the present work it was found necessary to add chromatography and thin layer chromatography was found very convenient for routine use

Normal values

The reference values in the papers published vary with the method being highest in the methods without purification The published reference values for adults correspond well with those published here During pregnancy there is an increase to 10–40 μg/l around the 4th week followed by a decrease to below 5 μg/l and a rise during the last weeks (32 33 37)

Normal values for children are mostly below 3 μg/l varying according to age and to the sensitivity and specificity of the methods Values are shown in Table 3 and few other papers (1 27 32)

17 OH P in CAH

Strott et al (31) determined the *secretion rate* of 17 OH P to be 113 and 55 mg/24 hrs in a five year-old and a sixteen year old patient with CAH—normal values in adults 1 1–1 9 mg/24 hrs Weinheimer et al (34) found values between 130 and 460 μg/l *in adrenal venous blood* in 10 patients with CAH (14–40 years)—values <100 μg/l in normal controls

As shown in Table 3 the values of 17 OH P in *peripheral blood* in untreated patients are found to be between 12 and 860 μg/l The values *rise after ACTH stimulation* two to five times or more and are *suppressed by decadron or cortisone treatment* These findings are in accordance with our experience as illustrated by the cases recorded here

In untreated patients 17 OH P will thus often be up to several hundred times the value in controls In extreme cases pregnanetriol may increase to 25 times the upper normal value and 17 KS up to 15 times This clinical sensitivity is valuable for the *diagnosis* of CAH (21 hydroxylase deficiency—and the differential diagnosis between this and other defects (19)) It is easier and faster to take a blood sample than to collect a 24 hour urine—especially in children in a poor condition or with adrenal insufficiency which should be treated immediately The value of 17 OH P in the differential diagnosis in precocious puberty and premature pubarche (5) is highest when it is studied during stimulation and if necessary steroid suppression

The usefulness of 17 OH P in the therapeutic *monitoring* of patients with CAH—if it is easier to make the fine adjustment with 17 OH P than it was with 17 KS and pregnanetriol—is being studied at present The value of cortisol and ACTH estimation is also being studied

The extent of the *diurnal variation* is essential for the timing of the blood sampling 17 OH P seems to vary like ACTH and cortisol In normal adults episodic secretion (0–3 μg/l) has been demonstrated (35) and integrated curves show a 24 hour variation of a similar size (0 8–1 7 μg/l) (13) Strott et al (31) found that the value at 8 p m was about 40% of the value at 8 a m (1 0 μg/l) and assumed that there would be no variation in patients with CAH In untreated patients and patients in poor control the highest values are found between 5 a m and 11 a m (>100 μg/l) with a fall during the day (to 40–90 μg/l at 6 p m) and a minimum at midnight (about 20 μg/l) followed by a new rise (3 16 20 21 36 38) In well treated patients there is no or a very small diurnal variation (16 20)

The relatively small diurnal variations found in normal adults and the large difference between normal values and the values in untreated patients whatever the time of day the sampling has been done indicates that diurnal variation does not injure the possibilities of diagnosis However the sampling time and especially the time which has passed since the last dose of cortisone has been taken may affect the value of 17 OH P in the therapeutic monitoring

Table 3 *Values for 17 OH P in untreated patients with CAH and reference values for children*

Ref no	Method	No	Age	Range (μg/l)	Reference values children (μg/l)
30+31	CPB +	5	5–16 y	40–220	
17	CPB ?	6	4 d –26 y	24–330	0 16–0 59 (5–11 y)
26	CPB +	5	7–18 y	146–222	
7	CPB O	7	6 d –5 y	46–194	<5
9	CPB O	7	<1 month >1 month	448 (mean) 423 (mean)	
23	CPB ?	23	10 d –23 y	40–≥400	≤20
4	CPB O	9	6–12 d	34–250	<10
10	RIA +	12	3 d –40 y	90–528	
25	RIA O	3		200–860	<3
21	CPB +	5	13 d –4 y	12–380	0 39 +/−0 22
20	RIA +	5	16 d –6 y	60–312	2 1 (<6 weeks) 0 3 (>6 weeks)
5	RIA +	2	6–7 y	2 56–13 57	0 03–1 37
19	RIA +	8	3 d –10 y	12–700	0 10–0 53
38	RIA +	6	3 d –3 y	180–612	1–6 3
12	CPB ?	7	2 d –3 y	90–380	<0 4
14	RIA (+)	4	?	37–365	0 17±0 23
16	RIA +	13	21 d –16 y	20–800	0 05–3 15
24	CPB ?	10	7 d –5 y	40–≥400	
6	RIA +	5	9–19 y		

O signifies *without* chromatography or other purification + signifies *with* chromatography or other purification

further during ACTH stimulation to fall again during cortisone treatment The 17 OH P increased more than tenfold after ACTH When treatment with cortisone was continued suppression was complete The diagnosis was CAH with a late debut

6 For 8 months this girl had developed pubic hair She was studied in the same way as patient no 5 and from the data shown and other studies she was shown to have a premature pubarche This patient is chosen for comparison and does *not* have CAH

DISCUSSION

Methods

Competitive protein binding technique (CPB) was first used in the determination of 17 OH P by Strott & Lipsett 1968 (29) later other methods were published (7 9 15 21 23 27 28) With the CPB technique preliminary purification is essential (although not always used) because of considerable interference from other steroids chiefly cortisol and progesterone In the examination of patients on cortisone treatment it is essential to avoid a determination of cortisol

Radioimmunoassays (RIA) which are more specific and sensitive have been predominant in recent years The necessity for purification in RIA depends on the specificity of the antibody but methods involving such purification must be considered the most precise Celite chromatography (2) paper chromatography (35) and sephadex (6 10 16 18 39) have been used Hami et al (14) used immunological purification with excess of antiserum before carrying out RIA

Until recently none of the published RIA methods for 17 OH P have shown the specificity of a direct analysis by comparing results with and without chromatografic purification Forest (11) used a highly specific antiserum in a method including celite chromatography and found comparable results with and without chromatography Values in pathological states are not given Tea et al (32) used a special technique by adding ^{3}H 17 OH P serving both as tracer for losses and as competitive steroid in immunological reaction After studying the consequences of omitting chro-

matography the authors conclude that chromatography must be used in all 17 OH P analysis

In the present work it was found necessary to add chromatography and thin layer chromatography was found very convenient for routine use

Normal values

The *reference values in the papers published* vary with the method being highest in the methods without purification The published reference values for adults correspond well with those published here During pregnancy there is an increase to 10–40 μg/l around the 4th week followed by a decrease to below 5 μg/l and a rise during the last weeks (32 33 37)

Normal values for children are mostly below 3 μg/l varying according to age and to the sensitivity and specificity of the methods Values are shown in Table 3 and few other papers (1 27 32)

17 OH P in CAH

Strott et al (31) determined the *secretion rate* of 17 OH P to be 113 and 55 mg/24 hrs in a five year-old and a sixteen year old patient with CAH—normal values in adults 1 1–1 9 mg/24 hrs Weinheimer et al (34) found values between 130 and 460 μg/l *in adrenal venous blood* in 10 patients with CAH (14–40 years)—values <100 μg/l in normal controls

As shown in Table 3 the values of 17 OH P in *peripheral blood* in untreated patients are found to be between 12 and 860 μg/l The values *rise after ACTH stimulation* two to five times or more and are *suppressed by decadron or cortisone treatment* These findings are in accordance with our experience as illustrated by the cases recorded here

In untreated patients 17 OH P will thus often be up to several hundred times the value in controls *In extreme cases* pregnanetriol may increase to 25 times the upper normal value and 17 KS up to 15 times This clinical sensitivity is valuable for the *diagnosis* of CAH (21 hydroxylase deficiency—and the differential diagnosis between this and other defects (19)) It is easier and faster to take a blood sample than to collect a 24 hour urine—especially in children in a poor condition or with adrenal insufficiency which should be treated immediately *The value of 17 OH P in* the differential diagnosis in precocious puberty and premature pubarche (5) is highest when it is studied during stimulation and if necessary steroid suppression

The usefulness of 17 OH P in the therapeutic *monitoring* of patients with CAH—if it is easier to make the fine adjustment with 17 OH P than it was with 17 KS and pregnane triol—is being studied at present The value of cortisol and ACTH estimation is also being studied

The extent of the *diurnal variation* is essential for the timing of the blood sampling 17 OH P seems to vary like ACTH and cortisol In normal adults episodic secretion (0–3 μg/l) has been demonstrated (35) and integrated curves show a 24 hour variation of a similar size (0 8–1 7 μg/l) (13) Strott et al (31) found that the value at 8 p m was about 40% of the value at 8 a m (1 0 μg/l) and assumed that there would be no variation in patients with CAH In untreated patients and patients in *poor control the highest values are found be*tween 5 a m and 11 a m (>100 μg/l) with a fall during the day (to 40–90 μg/l at 6 p m) and a minimum at midnight (about 20 μg/l) followed by a new rise (3 16 20 21 36 38) In well treated patients there is no or a very small diurnal variation (16 20)

The relatively small diurnal variations found in normal adults and the large difference between normal values and the values in untreated patients whatever the time of day the sampling has been done indicates that diurnal variation does not injure the possibilities of diagnosis However the sampling time and especially the time which has passed since the last dose of cortisone has been taken may affect the value of 17 OH P in the therapeutic monitoring

Table 3. *Values for 17 OH P in untreated patients with CAH and reference values for children*

Ref no	Method	No	Age	Range (µg/l)	Reference values children (µg/l)
30+31	CPB +	5	5–16 y	40–220	
17	CPB ?	6	4 d –26 y	24–330	0.16–0.59 (5–11 y)
26	CPB +	5	7–18 y	146–222	
7	CPB O	7	6 d –5 y	46–194	<5
9	CPB O	7	<1 month >1 month	448 (mean) 423 (mean)	
23	CPB ?	23	10 d –23 y	40–≥400	≤20
4	CPB O	9	6–12 d	34–250	<10
10	RIA +	12	3 d –40 y	90–528	
25	RIA O	3		200–860	<3
21	CPB +	5	13 d –4 y	12–380	0.39 +/–0.22
20	RIA +	5	16 d –6 y	60–312	2.1 (<6 weeks) 0.3 (>6 weeks)
5	RIA +	2	6–7 y	2.56–13.57	0.03–1.37
19	RIA +	8	3 d –10 y	12–700	0.10–0.53
38	RIA +	6	3 d –3 y	180–612	1–6.3
12	CPB ?	7	2 d –3 y	90–380	<0.4
14	RIA (+)	4	?	37–365	0.17±0.23
16	RIA +	13	21 d –16 y	20–800	0.05–3.15
24	CPB ?	10	7 d –5 y	40–≥400	
6	RIA +	5	9–19 y		

* O signifies *without* chromatography or other purification. + signifies *with* chromatography or other purification.

further during ACTH stimulation to fall again during cortisone treatment. The 17 OH P increased more than tenfold after ACTH. When treatment with cortisone was continued suppression was complete. The diagnosis was CAH with a late debut.

6. For 8 months this girl had developed pubic hair. She was studied in the same way as patient no. 5 and from the data shown and other studies she was shown to have a premature pubarche. This patient is chosen for comparison and does *not* have CAH.

DISCUSSION

Methods

Competitive protein binding technique (CPB) was first used in the determination of 17 OH P by Strott & Lipsett 1968 (29). Later other methods were published (7, 9, 15, 21, 23, 27, 28). With the CPB technique preliminary purification is essential (although not always used) because of considerable interference from other steroids, chiefly cortisol and progesterone. In the examination of patients on cortisone treatment, it is essential to avoid a co-determination of cortisol.

Radioimmunoassays (RIA), which are more specific and sensitive, have been predominant in recent years. The necessity for purification in RIA depends on the specificity of the antibody, but methods involving such purification must be considered the most precise. Celite chromatography (2), paper chromatography (35) and sephadex (6, 10, 16, 18, 39) have been used. Hami et al. (14) used immunological purification with excess of antiserum before carrying out RIA.

Until recently, none of the published RIA methods for 17 OH P have shown the specificity of a direct analysis by comparing results with and without chromatografic purification. Forest (11) used a highly specific antiserum in a method including celite chromatography and found comparable results with and without chromatography. Values in pathological states are not given. Tea et al. (32) used a special technique by adding ^{3}H 17 OH P, serving both as tracer for losses and as competitive steroid in immunological reaction. After studying the consequences of omitting chro-

durch Radioimmunologischen Nachweis von 17 α Hydroxyprogesteron Monatsschr Kinderheilkd 12? 6 1974
6 Simopoulos A P Marshall J R Delea C S & Bartter F C Studies on the deficien y of I hy droxylation in patients with congenital adrenal hyper plasia J Clin Endocrinol 3? 438 1971
7 Stewart Bentley M & Horton R 17 α hydroxy progesterone in human plasma J Clin Endocrinol 33 54? 1971
8 Stone S Kharma K M Nakamura R N Mishell D R & Thorneycroft I H A technique for the assay of 17 hydroxyprogesterone in serum using celite column chromatography and competitive pro tein binding Steroids 18 161 1971
?9 Strott C A & Lipsett M B Measurement of 17 hydroxyprogesterone in human plasma J Clin Endocrinol 28 14?6 1968
30 Strott C A Yoshimi T Bardin C W & Lipsett M B Blood progesterone and 17 hydroxyproges terone levels and production rates in a boy with viri lizing congenital adrenal hyperplasia J Clin Endocrinol 28 1085 1968
31 Strott C A Yoshimi T & Lipsett M B Plasma progesterone and 17 hydroxyprogesterone in normal men and children with congenital adrenal hyperplasia J Clin Invest 48 930 1969
3? Tea N T Castanier M Roger M & Scholler R Simultaneous radioimmunoassay of plasma proges terone and 17 hydroxyprogesterone normal values in children in men and in women throughout the men strual cycle and in early pregnancy J Steroid Biochem 6 1509 1975
33 Tulshinsky D & Simmer H H Sources of plasma 17 α hydroxyprogesterone in human pregnancy J Clin Endocrinol Metab 35 799 1972
34 Weinheimer B Oertel G W Leppla W Blaise H & Bette L Adrenal venous plasma concentration of 17 OH progesterone and urinary pregnanetriol excretion in 10 cases of adrenogenital syndrome (abstract) Acta Endocrinol (Kbh) Suppl 100 6? 1965
35 West C D Mahajan D K Chavre V J Nabors C J & Tyler F H Simultaneous measurement of multiple plasma steroids by radioimmunoassay dem onstrating episodic secretion J Clin Endocrinol Metab 36 1230 1973
36 West C D Stanchfield J B Atcheson J B Ralli son M L & Tyler F H Circadian variation in plasma ACTH and cortisol and its precursors in congenital adrenal hyperplasia (abstract) Clin Res 21 190 1973
37 Yoshimi T Strott C A Marshall J R & Lipsett M B Corpus luteum function in early preg nancy J Clin Endocrinol 29 ?25 1969
38 Youssefnejadian E & David R Early diagnosis of congenital adrenal hyperplasia by measurement of 17 hydroxyprogesterone Clin Endocrinol 4 451 1975
39 Youssefnejadian E Florensa E Collins W P & Sommerville I F Radioimmunoassay of 17 hy droxyprogesterone Steroids ?0 773 197?

Submitted Sept 13 1977
Accepted June ?5 1978

(K E P) Children s Hospital Fuglebakken
Drosselvej 57
2000 Copenhagen F
Denmark

We may conclude from the literature and the data here shown that the measurement of 17 OH P by a specific method such as that described has great importance for the diagnosis and perhaps also for the therapeutic monitoring of CAH (21 hydroxylase deficiency) Possibly it may also be used in the differential diagnosis in Cushing s syndrome During pregnancy there is a rather large variation in 17 OH P but it has been suggested (22) to use the analysis to show the presence of theca lutein cysts in patients with gestational trophoblastic neoplasms

REFERENCES

1 Abraham G E Hopper K Tulchinsky D Swerdloff R S & Odell W D Simultaneous measurement of plasma *progesterone* 17 hydroxyprogesterone & estradiol 17B by radioimmunoassay *Anal Letters 4* 325 1971
2 Abraham G E Swerdloff R S Tulchinsky D Hopper K & Odell W D *Radioimmunoassay* of plasma 17 hydroxyprogesterone *J Clin Endocrinol 33* 42 1971
3 Atherden S Barnes N D & Grant D B Circadian variation in plasma 17 hydroxyprogesterone in patients with congenital adrenal hyperplasia *Arch Dis Child 47* 602 1972
4 Atherden S M Edmunds A T & Grant D B Plasma 17 hydroxy*progesterone in* newborn infants with congenital adrenal hyperplasia and in infants with normal adrenal function *Arch Dis Child 49* 192 1974
5 August G P Hung W & Mayes D M Plasma androgens in premature pubarche Value of 17 hydroxyprogesterone in differentiation from congenital adrenal hyperplasia *J Pediatr 87 246 1975*
6 Bacon G E Spencer M L & Kelch R P Effect of cortisol treatment on hormonal relat*ionships in* congenital adrenal hyperplasia *Clin Endocrinol 6* 113 1977
7 Barnes N D & Atherden S M Diagnos*is of* congenital adrenal hyperplasia by measurement of plasma 17 hydroxyprogesterone *Arch Dis Child 47* 62 1972
8 Erlanger B F Borek P Beiser S M & Lieberman S *J Biol Chem 228* 713 1958
9 Franks R C Screening tests for congenital adrenal hyperplasia *J Clin Endocrinol Metab 35 831 1972*
0 Franks R C Plasma 17 hydroxyprogesterone 21 deoxycortisol and cortisol in congenital adrenal hyperplasia *J Clin Endocrinol Metab 39* 1099 1974
1 Forest M G Use of highly specific antibodies against 17 OH progesterone in a simplified non chromatographic RIA and in the simultaneous determination of four sex hormones in human plasma *Horm Res 7* 260 1976
12 Gillet P Interet des dosages plasmatiques d'activite renine testostérone 17 alpha hydroxyprogesterone et A C T H dans le diagnostic et la surveillance de hyperplasies congenitales des surrenales par deficit en 21 hydroxylase Lyon 1975 Thesis
13 Gutai J P Meyer W J Kowarski A A & Migeon C J Twenty four hour integrated concentrations of progesterone 17 hydroxyprogesterone and cortisol in normal male subjects *J Clin Endocrinol Metab 44* 116 1977
14 Hami M Rosler A & Rabinowitz D A non chromatographic radioimmunoassay for 17 alpha hydroxyprogesterone *J Clin Endocrinol Metab 40* 863 1975
15 Holmdahl T H & Johansson E D B Peripheral plasma levels of 17 α hydroxyprogesterone progesterone and oestradiol during normal menstrual cycle in women *Acta Endocrinol (Kbh) 71* 743 1972
16 Hughes I A & Winter J S D The application of a serum 17 OH progesterone radioimmunoassay to the diagnosis and management of congenital adrenal hyperplasia *J Pediatr 88* 766 1976
17 Jenner M R Grumbach M M & Kaplan S L Plasma 17 OH progesterone in maternal and umbilical cord plasma in children and in congenital adrenal hyperplasia (abstract) *Pediatr Res 4* 380 19 0
18 Janne O Apter D & Vikho R Assay to testosterone progesterone and 17 α hydroxyprogesterone in human plasma by radioimmunoassay after separation in hydroxyalkoxypropyl sephadex *J Steroid Biochem 5* 155 1974
19 Janne O Perheentupa J Viinikka L & Vihko R Plasma pregnenolone progesterone 17 hydroxyprogesterone testosterone and 5-α-dihydrotestosterone in different types of congenital adrenal hyperplasia *Clin Endocrinol 4* 39 1975
20 Lippe B M LaFranchi S H Lavin N Parlow A Coyotupa J & Kaplan S A Serum 17-α hydroxyprogesterone progesterone estradiol and testosterone in the diagnosis and management of congenital adrenal hyperplasia *J Pediatr 85* 182 1974
21 Loras B Roux H Audi Parera L David M & Bertrand J 17-α hydroxyprogestererone plasmatique dans l hyperplasie congénitale des surrenales par *déficit en 21* hydroxylase traitee et non traitée *Biomedicine 21* 317 1974
22 Osathanondh R Goldstein D P Tulchinsky D & Finn A E Serum 17 α hydroxyprogesterone in patients with gestational trophoblastic neoplasms *Obstet Gynaecol 49* 77 1977
23 Pham Huu Trung M T Gourmelen M & Girard F The simultaneous assay of cortisol and 17 α hydroxyprogesterone in the plasma of patients with congenital adrenal hyperplasia *Acta Endocrinol (Kbh) 74* 316 1973
24 Pham Huu Trung M T Raux M C Gourmelen M Baron M C & Girard F Plasma aldosterone concentrations related to 17 α hydroxyprogesterone in congenital adrenal hyperplasia *Acta Endocrinol (Kbh) 82* 572 1976
25 Schnakenburg K V Bidlingmaier F & Knorr D Diagnostik und Verlaufskontrolle des adrenogenitalen Syndroms vom Typ des 21 Hydroxylasedefektes

Acta Pædiatr Scand 68 213–217 1979

INCREASE IN BILIRUBIN BINDING TO ALBUMIN WITH CORRECTION OF NEONATAL ACIDOSIS

KIYOSHI KOZUKI WILLIAM OH JOHN WIDNESS and WILLIAM J CASHORE

From the Section of Reproductive and Developmental Medicine Brown University Program in Medicine and Department of Pediatrics Women and Infants Hospital of Rhode Island Providence Rhode Island USA

ABSTRACT Kozuki K Oh W Widness J and Cashore W (Department of Pediatrics Women and Infants Hospital Providence Rhode Island USA) Increase in bilirubin binding to albumin with correction of neonatal acidosis Acta Paediatr Scand 68 213 1979.—Twenty six serial measurements of free bilirubin concentration and apparent association constant of bilirubin for albumin (Ka) at a bilirubin albumin molar ratio of 0 8 were performed and compared with baseline values in 11 newborn infants with acidosis before treatment and during recovery from acidosis When arterial pH was corrected from 7 12±0 02 (Mean±S E M) to 7 34±0 02 there was a significant decrease in serum free bilirubin concentration and a significant increase in the Ka at molar ratio 0 8 The data offer *in vivo* evidence that correction of acidosis in the neonate results in an improvement of the apparent bilirubin binding affinity of albumin

KEY WORDS Newborn bilirubin binding albumin acidosis

Neonatal acidosis is associated with an increased risk of bilirubin encephalopathy (kernicterus) Stern et al (15) reported an increased incidence of kernicterus in small sick premature infants following acidosis and hypothermia Gartner et al (6) and Keenan et al (9) have reported similar findings in sick preterm infants Animal studies have shown increased bilirubin uptake in the brains of guinea pigs subjected to respiratory acidosis (4) and of Rhesus monkeys asphyxiated at birth (10) *In vitro* Odell and associates have observed a shift in the spectral curve of bilirubin from the bound toward the unbound state at lowered pH (13) Nelson et al however using a cell culture model reported that acidosis affects the distribution and toxicity of bilirubin by increasing the deposition of bilirubin in cells rather than by decreasing the affinity of albumin for bilirubin (12) In the current study we have measured bilirubin–albumin association constants in 11 newborn infants during and after recovery from acidosis to evaluate the effect of acidosis on bilirubin binding to albumin in neonatal sera

MATERIAL AND METHODS

The subjects were 11 newborn infants admitted to the Newborn Special Care Unit of the Women and Infants Hospital of Rhode Island with respiratory distress and acidosis The primary diagnoses included respiratory distress syndrome in 6 and birth asphyxia in 5 infants complications included pneumothorax in 2 infants aspiration pneumonia in 2 seizures in 2 and hemorrhagic shock in one infant Nine of the 11 infants survived The average birth weight was 2 8 0 g (range 1530–5 75) and the average gestational age was 36 6 weeks (range 31–42) The criterion for admission to the study was an initial arterial blood pH of less than 7 25 At the time of the first and subsequent arterial pH determinations (the 11 infants had a total of 6 follow up measurements) 0 5 ml of unheparinized blood was drawn from the arterial catheter for the bilirubin binding studies Specimens were obtained at the time of each pH determination until the acidosis was corrected to a pH value above 7 30

For the correction of acidosis the patients received sodium bicarbonate according to the formula

Base deficit (mEq/l)×body wt (kg)×0 3=mEq sodium bicarbonate given

The 0 9 M bicarbonate was diluted 1 1 with sterile water and was given over 30–60 minutes this infusion of bicarbonate was repeated once if the metabolic acidosis persisted In addition positive pressure assisted ventilation was required in 5 infants who had combined respiratory and metabolic acidosis (2) apneic spells (1) or persistent hypoxemia despite application of continous positive air way pressure (2)

Free bilirubin (nmol/l) B/A=0 8 pH 7 4 T=34 C	$Ka\times10$ M
1 ±5	0 95±0 08
11±4	1 29±0 10
0 005	<0 01

an increase in apparent bilirubin albumin association constant (Ka) from $0\ 95\pm0\ 08\times10^{8}$ M^{-1} to $1\ 29\pm0\ 1\times10^{8}$ M^{-1} ($p<0\ 01$) Rectal temperature averaged 98 1±0 02 F at the time the studies were begun and remained stable throughout the period of study Serum glucose was 54±7 mg/100 ml at the beginning of the study one value was 24 mg/100 ml and the remaining patients had serum glucose values in excess of 37 mg/100 ml

As shown in Fig 1 increases in blood pH were associated with significant decreases ($r=0\ 640$ $p<0\ 005$) in free bilirubin (i e after dilution of serum and addition of bilirubin to a B/A ratio of 0 8) and significant increases in apparent Ka ($r=0\ 627$ $P<0\ 005$) Reduction in base deficit was associated with a significant decrease in free bilirubin and significant increase in Ka ($r=0\ 473$ $p<0\ 02$ for both correlations (graphs not shown)) There was no correlation between changes in free bilirubin or Ka and changes in postnatal age time after entry into the study P_{o} or P_{r} Acidosis recurred in two patients after early correction and in both this recurrence was accompanied by a decrease in Ka

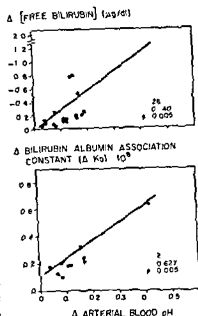


Fig 1 Decrease in free bilirubin concentration (upper part) and increase in bilirubin–albumin association constant (lower part) with increasing arterial blood pH during correction of neonatal acidosis serial observations in 11 infants There are 26 comparisons since multiple repeat determinations were made in 6 patients Free bilirubin was measured under the following conditions pH 7 4 T=34°C and bilirubin albumin molar ratio=0 8 after dilution of serum and addition of bilirubin

DISCUSSION

Acidosis was associated with a reduction in apparent bilirubin binding affinity even when the free bilirubin assays were performed at pH 7 4 Using Sephadex gel filtration at a constant pH of 7 4 we have previously shown that the bilirubin binding capacity is higher in healthy clinically stable infants than in those with neonatal acidosis and respiratory distress (2 3) In those studies sequential changes in arterial blood pH were not documented in relation to changes in bilirubin binding characteristics an association between changes in acid–base balance and changes in bilirubin binding is shown in the current report

Using the peroxidase method Nelson et al (12) and Jacobsen & Brodersen (7) have found that *in vitro* the bilirubin binding to albumin is not influenced by changes in pH at the range of 7 to 9 3 The chemical structure of bilirubin itself also precludes the possibility that its high affinity binding to the albumin molecule would be affected by hydrogen ion concentration (1)

When Odell et al (13) lowered pH of their reaction medium from 7 4 to 6 8 *in vitro* a shift was observed in the spectral curve of the

Table 1 *Acid–base albumin and bilirubin values in 11 newborn infants (26 determinations) befo and after correction of acidosis*

	Age (hrs)	Arterial blood pH	P_{O_2} (mmHg)	P_{CO_2} (mmHg)	Base deficit (mEq/l)	Albumin (g/100 ml)	Indirect Bilirubin (mg/100 ml)
Before correction	9 6±2 3	7 12±0 02	64 4±8 3	34 1±4 7	−17 6±2 2	3 35±0 0	1 9±0 5
After correction	18 5±3 5	7 34±0 02	84 3±11	28 9±3 9	−7 8±1 7	3 05±0 15	2 8±0 2
p[a]	–	–	n s	n s	<0 001	n s	<0 05

All values are $M\pm S$ E M
[a] Paired *T* test
Values for free bilirubin and apparent *Ka* obtained at B/A molar ratio=0 8 pH 7 4 and 24 C after dilution of serum and addition of bilirubin

Serum albumin and total and direct bilirubin concentrations were determined in duplicate by standard methods (5 11) precision of the methods was ±0 1 g/100 ml at 4 g/100 ml for albumin (coefficient of variation=2 5±1%) and ±0 3 mg/100 ml at 10 mg/100 ml for bilirubin determinations (coefficient of variation 3±2%) Free bilirubin concentrations after dilution of the serum (25 microliters in 1 0 ml of buffer) and addition of crystalline bilirubin to achieve the desired bilirubin albumin molar ratio were determined in duplicate by the horseradish peroxidase method (8) with a precision of ±1 nmol/l at 25 nmol/l (coefficient of variation 4±2%) Aliquots of 25 microliters of serum were used for each free bilirubin determination and the assays were carried out in 1 0 ml of 1/15 M sodium phosphate buffer pH 7 4 at 24°C To correct for postnatal changes in serum bilirubin and albumin concentration the bilirubin to albumin molar ratio of each serum was raised to 0 8 by addition of crystalline bilirubin (Sigma) in 0 1 NaOH and the free bilirubin assay was performed at that concentration The apparent association constant *Ka* was calculated from the law of mass action

$$Ka=\frac{(BA)}{(b)\times(a)}$$

in which Ka=the apparent association constant of bilirubin for albumin (BA)=the molar concentration of the bilirugin–albumin complex (b)=the molar concentration of free bilirubin and (a)=the molar concentration of albumin without bound bilirubin

At a bilirubin albumin ratio of 0 8 bilirubin would be bound to 80% (0 8) of its primary binding sites on albumin while 20% (0 2) of the albumin sites would contain no bilirubin giving relative concentrations of 0 8 for bound bilirubin (BA) and 0 2 for albumin without bound bilirubin (a)

The molecular weight of bilirubin was assumed to be 585 of albumin 69 000 and the indirect was assumed to equal the bound bilirubin concentration

In 8 samples taken from 4 of the 11 study infants sufficient sample aliquots were obtained to analyze the apparent bilirubin albumin association constant using both the molar ratio formula above and Scatchard graph analysis in the same specimen the results showed good agreement between the two methods for the determinations of Ka which averaged 0 85±0 08×10 M^{-1} ($M\pm$S E M) by Scatchard plot and 0 93±0 10×10 M^{-1} by direct calculation from the formula

Changes in free bilirubin concentrations and association constants were analyzed by paired *t* test Relationships between changes in free bilirubin or Ka and changes in postnatal age pH P_{CO_2} or base deficit were determined by regression analysis

RESULTS

A total of 26 serial determinations of arterial blood gas and acid–base values free bilirubin and apparent association constant were made during correction of acidosis in the 11 infants and were compared with baseline values Pre and post-correction acid–base values and blood gas tensions are shown in Table 1 When first studied at an average age of 9 6±2 3 hours ($M\pm S$ E M) the infants had an average arterial blood pH of 7 12±0 02 Correction of acidosis to pH 7 34±0 02 was achieved by 18 5±3 5 hours of age Correction of acidosis was accompanied by an increase in P_{O_2} a slight fall in P_{CO_2} and a change of +9 8 mEq/l in the base deficit From the beginning of the study until the time of pH correction serum albumin concentration did not change significantly (3 35±0 02 to 3 05±0 15 g/100 ml) there was an increase in indirect bilirubin concentration (1 9±0 5 to 2 8±0 2 mg/100 ml) At a bilirubin to albumin molar ratio of 0 8 correction of acidosis was accompanied by a significant decrease in free bilirubin concentration from 42±5 to 31±4 nmol/l (p<0 005) and

Kernicterus in asphyxiated newborn rhesus monkeys *Exp Neurol 9* 43 1964
11 Martinek R G Improved micromethod for determination of serum bilirubin *Clin Chim Acta 13* 161 1966
1 Nelson T Jacobsen J & Wennberg R P Effect of pH on the interaction of bilirubin with albumin and tissue culture cells *Pediatr Res 8* 963 1974
13 Odell G B Cohen S N & Kelly P C Studies in kernicterus II The determination of the saturation of serum albumin with bilirubin *J Pediatr 74* 14 1969
14 Odell G B Cukier J O Ostrea E M Jr et al The influence of fatty acids on the binding of bilirubin to albumin *J Lab Clin Med 89* 95 1977
15 Stern L Doray B Chan G & Schiff D Bilirubin metabolism and the induction of kernicterus In Bergsma D and Blondheim S H (eds) *Bilirubin metabolism in the newborn (II)* American Elsevier Publ Co Inc New York 1976 pp 255-63

Submitted April 7 1978
Accepted June 20 1978

(W O) Department of Pediatrics
Women and Infants Hospital of Rhode Island
50 Maude Street
Providence Rhode Island
USA 02908

bilirubin–albumin complex with a decrease in absorbance at 460 nm this spectral shift was initially interpreted as suggesting dissociation of the complex (13) However in a more recent study (14) the same workers have postulated a shift of bilirubin from primary to secondary binding sites under the influence of strong competitors (e g fatty acids) resulting in spectral changes not necessarily related to a release of bilirubin Other investigators have also interpreted these spectral changes as representing pH induced alterations in the spectrum of the complex rather than increased dissociation of bilirubin from albumin (7 12) In the present studies all binding assays were performed at the same pH so that the increases in free bilirubin and the decreases in the bilirubin–albumin association constant associated with neonatal acidosis were not effects of hydrogen ion concentration alone It is of interest that the increment in the binding of bilirubin to albumin with the correction of acidosis was associated with a significant reduction in the base deficit (Table I) One may speculate that acidosis is associated with the presence of endogenous anions (which may themselves contribute to metabolic acidosis) capable of displacing bilirubin from its albumin binding sites and thereby increasing plasma free bilirubin With correction of acidosis a concomitant improvement in the circulatory and metabolic status of the infants may reduce the effects of displacers by enhanced clearance reduction in their formation or changes in the affinity of anions other than bilirubin for albumin binding sites

To control the reaction rate and molar ratio and to make use of small aliquots of serum free bilirubin concentrations in these studies were determined at 24°C after dilution of the serum and addition of bilirubin the values reported for the apparent association constants were obtained under those conditions Estimation of free bilirubin and *Ka* at 37°C in undiluted serum is technically more difficult and would require a much larger serum sample (a limiting factor in the study of small infants) but if technically feasible would more nearly approximate *in vivo* conditions

The concentration of free bilirubin which is toxic to the human central nervous system *in vivo* has not been determined Acidosis appears to be associated with an increase in the free bilirubin concentration furthermore acidosis also enhances the deposition of free bilirubin in the tissues and/or increases the susceptibility of the tissues to bilirubin toxicity as shown *in vitro* in several studies (4 10 12 13) Avoidance of or prompt correction of acidosis in jaundiced newborns appears important to maintain the optimal albumin binding characteristics and to protect the potentially susceptible tissue sites

REFERENCES

1 Brodersen R Prevention of kernicterus based on recent progress in bilirubin chemistry *Acta Paediatr Scand 66 625 1977*

2 Cashore W J Horwich A Karotkin E H & Oh W The influence of gestational age and clinical status on bilirubin binding capacity in newborn infants determined by Sephadex G 25 gel filtration technique *Am J Dis Child 131 898 1977*

3 Cashore W J Horwich A & Oh W Relationship of serum total bilirubin binding capacity to infant's maturity and clinical status *Pediatr Res 10* 4?? 1976 (Abstract)

4 Diamond I & Schmid R Experimental bilirubin encephalopathy The mode of entry of bilirubin-¹⁴C into the central nervous system *J Clin Invest 46* 678 *1966*

5 Doumas B T Watson W A & Biggs H G Albumin standards and the measurement of serum albumin with bromcresol green *Clin Chim Acta 31* 87 1971

6 Gartner L M Snyder R N Chabon R H & Bernstein J Kernicterus high incidence in prematures with low serum bilirubin concentrations *Pediatrics 45 906 1970*

7 Jacobsen J & Brodersen R The effect of pH on albumin–bilirubin binding affinity In Bergsma D & Blondheim S H (eds) *Bilirubin metabolism in the newborn (II)* American Elsevier Publ Co Inc New York *1976 pp 175–178*

8 Jacobsen J & Wennberg R P Determination of unbound bilirubin in the serum of newborns *Clin Chem 20 783 1974*

9 Keenan W J Perlstein P H Light I J & Sutherland J M *Kernicterus in small sick infants receiving phototherapy* *Pediatrics* 49 652 1972

10 Lucey J F Hibbard E & Behrman R E et al

kernıcterus ın asphyxıated newborn rhesus monkeys *Exp Neurol* 9 43 1964
Martınek R G Improved mıcromethod for deter mınatıon of serum bılırubın *Clın Chım Acta* 13 161 1966
Nelson T Jacobsen J & Wennberg R P Effect of pH on the ınteractıon of bılırubın wıth albumın and tıssue culture cells *Pedıatr Res* 8 963 1974
Odell G B Cohen S N & Kelly P C Studıes ın kernıcterus II The determınatıon of the saturatıon of serum albumın wıth bılırubın *J Pedıatr* 74 14 1969
Odell G B Cukıer J O Ostrea E M Jr et al The ınfluence of fatty acıds on the bındıng of bılırubın to albumın *J Lab Clın Med* 89 295 1977
15 Stern L Doray B Chan G & Schıff D Bılırubın metabolısm and the ınductıon of kernıcterus In Bergsma D and Blondheım S H (eds) *Bılırubın metabolısm ın the newborn (II)* Amerıcan Elsevıer Publ Co Inc New York 1976 pp 255–263

Submıtted Aprıl 7 1978
Accepted June 20 1978

(W O) Department of Pedıatrıcs
Women and Infants Hospıtal of Rhode Island
50 Maude Street
Provıdence Rhode Island
USA 0 908

Acta Pædiatr Scand 68 219–223 1979

BENIGN PAROXYSMAL TORTICOLLIS IN INFANCY

GUNNAR SANNER and BJÖRN BERGSTRÖM

From the Departments of Paediatrics and Otolaryngology Central Hospital Karlstad Sweden

ABSTRACT Sanner G and Bergstrom B (Departments of Paediatrics and Otolaryngology Central Hospital Karlstad Sweden) Benign paroxysmal torticollis in infancy Acta Paediatr Scand 68 219 1979 —Benign paroxysmal torticollis is a condition characterized by attacks of head tilting often accompanied by lateral curvature of the trunc The first onset of these attacks usually occurs during the first months of life and they recur with a remarkable periodicity for 6–12 months after which time they gradually subside in intensity and frequency As a rule the attacks have ceased completely before the age of 2 years but in some cases they continue in a modified form as attacks of ataxia The aetiology is unknown and there is no evidence of bilateral peripheral vestibular disturbances as has been suggested in earlier reports The ataxia seen in some cases rather suggests a dysfunction of the cerebellum or of the vestibulo-cerebellar connections Four own patients with this syndrome are presented and discussed

KEY WORDS Paroxysmal torticollis infancy periodic disorders paroxysmal ataxia

In 1969 Snyder (14) reported 12 children with a paroxysmal condition appearing in infancy and characterized by periods of torticollis like posture of the head

A further case was reported by Gourley (8) Chutorian (3) presented 5 children with the same condition and added to the clinical picture the occurrence of lateral curvature of the trunc Two of the children also showed an abnormal posture on vertical suspension No further reports of this condition seem to have appeared in the literature and the condition is not to be found in ordinary textbooks of pediatrics or child neurology

In 1976 the authors presented two children with paroxysmal torticollis at the 18th Scandinavian Pediatric Congress (13) Since then we have recognized two further cases and will present our experiences from 4 children with this peculiar condition

CASE HISTORIES

Case 1

J N Female First and only child of healthy parents who were not consanguineous Her paternal grandfather suffered from a torticolli[illegible] life Her father had a torticollis from early infancy to 1 5 years of age and according to his mother it had been of the same type as in J N Her pre   pen and neonatal history was normal as well as her psychomotor development

From the age of one month attacks appeared when the girl kept her head mostly to the left side but also to the right Her head was also slightly retracted When lying prone the baby could move her head freely In other positions straightening of her neck caused irritation Otherwise the baby seemed to be perfectly well during the attacks The duration of the attacks were 3–7 days with a maximum of head tilting on the second day after which the condition successively subsided The attacks were recognized by her parents when the baby woke up in the morning

During the first 6 months the attacks recurred very regularly with a free interval of 14 days Later on the frequency diminished as well as the severity of the attacks Treatment with carbamazepine was tried but had no effect The last attack was seen at 13 months of age The girl is now 2 5 years old and is healthy and well developed in all respects

Examinations Physical and neurological examinations at several occasions revealed no abnormalities except for the torticollis An ophthalmological examination was normal as was an electroencephalogram

Oto-neurological examination including electronystagmography with caloric tests in a free interval revealed no abnormalities Repeated hearing tests the latest at 2 5 years of age were normal

Case 2

J H Female The third of three children of healthy parents who were not consanguineous A brother of J H



Fig 2 Paroxysmal torticollis in case ? at 9 months of age showing curvature of the trunc and extension of the right leg (Ink drawing from 8 mm movie)

recognized the condition in the morning At the onset the baby seemed to sweat and was *irritable but showed* no other signs of distress during the attacks At examination during an attack the baby was at first seen sleeping supine with her head bent to the left Awaking she bent her head to the right and retracted it Slight curvation of the trunc but no change of the tonus in her arms or legs was noticed The position was accentuated when the girl was held upright

The girl is now 8 months old and except for the regularly recurring attacks healthy and well developed

Ex m nations Physical and neurological examinations were normal Roentgen stud es of the spine electroencephalography during an attack and echo-encephalography revealed no abnormalities ENG Normal caloric reactions

DISCUSSION

The attacks of head tilting in these four cases evidently represent the same condition as was reported by Snyder (14) Gourley (8) and Chutorian (3) The main clinical characteristics of the condition according to this series and reports from the literature are shown in Table 1

Table 1 *Main clinical features of benign paroxysmal torticollis according to cases reported*

Paroxysmal occurrence of head tilting and—retraction
Often lateral curvature of the trunc
Sometimes extension pattern of one leg
Usually more abnormal posture in supine and upright positions
Sometimes distress and abnormal rolling of the eyes at onset of attacks
Regular periodicity of attacks for a long time
Duration of attacks hours–days
Onset within the first year of life
Recovery from 1 year to 5 years of age
Female preponderance

In more severe cases (case 2) extension of the extremities on one side are also seen *This involvement of the extremities* has not been reported before

In some cases the onset of an attack is *accompanied by distress and vomiting* The first attack usually occurs during the first year of life and we have even seen it during the first week (case 4) *The age of recovery* is from 1 year to 5 years according to Snyder (14) The duration of attacks varies from 10 min to several days

The main diagnostic problem is the first attack appearing in a previously healthy infant Torticollis in infancy may be *the first* sign of a posterior fossa tumor (6) which therefore must be ruled out especially when accompanied by vomiting

Recurrent cervical dislocation as well as *epilepsy has been* discussed (3 14) but never proved Electroencephalography also during attacks in 2 of our cases showed no abnormality However this does not discard epileptic discharge at a lower subcortical *level* as a cause of paroxysmal torticollis Therefore we treated *2 of our patients* (cases 1 and 2) with carbamazepine which has had excellent effect in spinal seizures (5) as well as in paroxysmal choreo athetosis (16) but this



Fig 1 Paroxysmal torticollis in case 2 at 8 months of age (*a*) to the left side and (*b*) at the next occasion to the right

has a leftsided sensorineural hearing loss Several other relatives also have sensorineural hearing loss

Pre peri and neonatal history as well as psychomotor development was quite normal From 3 months of age attacks were noted when the girl kept her head to one side (Fig 1) At the same time her trunc was curvated and her contralateral leg rigidly extended with plantar flexion of the foot (Fig 2) This posture was most pronounced in upright and supine positions but inhibited when prone or crawling

At the onset of some attacks the mother noticed that the girl s eyes rolled upwards At one occasion when her neck was straightened rolling of her eyes occurred until her head was tilted again No engagement of the face and mouth was noted

The attacks were always recognized in the morning Especially at the beginning of an attack the girl sometimes seemed unwell irritated and drowsy

The duration of the attacks varied from 3–12 hours The attacks recurred very regularly once a month but from the age of 1 year the frequency gradually diminished From 1 5 to 2 5 years of age she had about 7 attacks some very mild but other severe and showing some additional signs The latest attack was described as follows She had a balance disturbance and often fell She walked on a broad base unsteady with short steps She had only a slight torticollis and no affection of her trunc or leg When moving she preferred to crawl with her head bent down Her favourite position was crouching An evident tremor of grasping was noticed Her eyes were seen rolling up at occasions During the latest attack lasting 12 hours she refused to eat and drink She vomited twice and a few times she complained of headache This attack as the others stopped suddenly as a light button is switched off She then could walk drink eat and play as usual The girl is now 2 5 years old and quite healthy and well developed

Examinations Physical and neurological examinations never revealed any abnormalities Ophthalmological examination electroencephalography and echo-encephalography were normal as was electrocardiography during an attack Chromatography of the urine showed no abnormal organic acids Roentgen studies of the skull and cervical spine was normal Caloric tests during attack and in a free interval showed normal reactions bilaterally registered by ENG Hearing tests indicate normal hearing

Case 3

M J Female First and only child The parents were not consanguineous Her mother has a slight sensorineural hearing loss on one side Pre peri and neonatal history was normal From 2 months of age attacks with the head tilted to the left and sometimes slight curvation of the trunc During an attack the torticollis gradually appeared within 2 days and faded within 2 weeks During the attacks the baby was quite well The attacks occurred very regularly with a free interval of 14±1 days Gradually the attacks became less visible and were noticed only when she was tired From 1 5 years of age no attacks were seen The girl is now 2 years old healthy and well developed

Examinations Physical and neurological examination was normal Ophthalmological examination and electroencephalography during an attack showed normal results Roentgen studies of the cervical spine revealed no abnormalities Rotation test showed normal reactions (The girl refused to cooperate in caloric tests) Hearing tests were normal

Case 4

A K B Female First and only child of healthy parents who were not consanguineous The prenatal period was normal Because of a prolonged labour the child was delivered by vacuum extraction The apgar score was 8 at 1 min Two hours after birth two short convulsion episodes were observed otherwise the neonatal period was normal

At 1 week of age a torticollis was noticed which disappeared within 1 week Thereafter especially rightsided torticollis attacks reappeared regularly with a free interval of 16–19 days and a duration of 6–8 days The parents



Fig 2 Paroxysmal torticollis in case ? at 9 months of age showing curvature of the trunc and extension of the right leg (Ink drawing from 8 mm movie)

recognized the condition in the morning At the onset the baby seemed to sweat and was irritable but showed no other signs of distress during the attacks At examina tion during an attack the baby was at first seen sleeping supine with her head bent to the left Awaking she bent her head to the right and retracted it Slight curvation of the trunc but no change of the tonus in her arms or legs was noticed The position was accentuated when the girl was held upright

The girl i now 8 months old and except for the re gularly recurring attacks healthy and well developed

Exam nations Physical and neurological examinations were normal Roentgen studies of the spine electroence phalography during an attack and echo-encephalography revealed no abnormal ties ENG Normal caloric reac tions

DISCUSSION

The attacks of head tilting in these four cases evidently represent the same condition as was reported by Snyder (14) Gourley (8) and Chu tonan (3) The main clinical characteristics

Table 1 *Main clinical features of benign par oxysmal torticollis according to cases re ported*

Paroxysmal occurrence of head tilting and—retraction
Often lateral curvature of the trunc
Sometimes extension pattern of one leg
Usually more abnormal posture in supine and upright positions
Sometimes distress and abnormal rolling of the eyes at onset of attacks
Regular periodicity of attacks for a long time
Duration of attacks hours–days
Onset within the first year of life
Recovery from 1 year to 5 years of age
Female preponderance?

of the condition according to this series and reports from the literature are shown in Table 1

In more severe cases (case 2) extension of the extremities on one side are also seen This involvement of the extremities has not been reported before

In some cases the onset of an attack is accompanied by distress and vomiting The first attack usually occurs during the first year of life and we have even seen it during the first week (case 4) The age of recovery is from 1 year to 5 years according to Snyder (14) The duration of attacks varies from 10 min to several days

The main diagnostic problem is the first attack appearing in a previously healthy in fant Torticollis in infancy may be the first sign of a posterior fossa tumor (6) which therefore must be ruled out especially when accompanied by vomiting

Recurrent cervical dislocation as well as epilepsy has been discussed (3 14) but never proved Electroencephalography also during attacks in 2 of our cases showed no abnor mality However this does not discard epi leptic discharge at a lower subcortical level as a cause of paroxysmal torticollis Therefore we treated 2 of our patients (cases 1 and 2) with carbamazepine which has had excellent effect in spinal seizures (5) as well as in paroxysmal choreo athetosis (16) but this



Fig 1 Paroxysmal torticollis in case 2 at 8 months of age (*a*) to the left side and (*b*) at the next occasion to the right

has a leftsided sensorineural hearing loss Several other relatives also have sensorineural hearing loss

Pre peri and neonatal history as well as psychomotor development was quite normal From 3 months of age attacks were noted when the girl kept her head to one side (Fig 1) At the same time her trunc was curvated and her contralateral leg rigidly extended with plantar flexion of the foot (Fig 2) This posture was most pronounced in upright and supine positions but inhibited when prone or crawling

At the onset of some attacks the mother noticed that the girl s eyes rolled upwards At one occasion when her neck was straightened rolling of her eyes occurred until her head was tilted again No engagement of the face and mouth was noted

The attacks were always recognized in the morning Especially at the beginning of an attack the girl sometimes seemed unwell irritated and drowsy

The duration of the attacks varied from 3–12 hours The attacks recurred very regularly once a month but from the age of 1 year the frequency gradually diminished From 1 5 to 2 5 years of age she had about 7 attacks some very mild but other severe and showing some additional signs The latest attack was described as follows She had a balance disturbance and often fell She walked on a broad base unsteady with short steps She had only a slight torticollis and no affection of her trunc or leg When moving she preferred to crawl with her head bent down Her favourite position was crouching An evident tremor of grasping was noticed Her eyes were seen rolling up at occasions During the latest attack lasting 12 hours she refused to eat and drink She vomited twice and a few times she complained of headache This attack as the others stopped suddenly as a light button is switched off She then could walk drink eat and play as usual The girl is now 2 5 years old and quite healthy and well developed

Examinations Physical and neurological examinations never revealed any abnormalities Ophthalmological examination electroencephalography and echo-encephalography were normal as was electrocardiography during an attack Chromatography of the urine showed no abnormal organic acids Roentgen studies of the skull and cervical spine was normal Caloric tests during attack and in a free interval showed normal reactions bilaterally registered by ENG Hearing tests indicate normal hearing

Case 3

M J Female First and only child The parents were not consanguineous Her mother has a slight sensorineural hearing loss on one side Pre peri and neonatal history was normal From 2 months of age attacks with the head tilted to the left and sometimes slight curvation of the trunc During an attack the torticollis gradually appeared within 2 days and faded within 2 weeks During the attacks the baby was quite well The attacks occurred very regularly with a free interval of 14±1 days Gradually the attacks became less visible and were noticed only when she was tired From 1 5 years of age no attacks were seen The girl is now 2 years old healthy and well developed

Examinations Physical and neurological examination was normal Ophthalmological examination and electroencephalography during an attack showed normal results Roentgen studies of the cervical spine revealed no abnormalities Rotation test showed normal reactions (The girl refused to cooperate in caloric tests) Hearing tests were normal

Case 4

A K B Female First and only child of healthy parents who were not consanguineous The prenatal period was normal Because of a prolonged labour the child was delivered by vacuum extraction The apgar score was 8 at 1 min Two hours after birth two short convulsion episodes were observed otherwise the neonatal period was normal

At 1 week of age a torticollis was noticed which disappeared within 1 week Thereafter especially rightsided torticollis attacks reappeared regularly with a free interval of 16–19 days and a duration of 6–8 days The parents

that all these children really suffered from a unilateral peripheral vestibular disorder Apparently ENG was not used and admittedly it is not easy to properly evaluate nystagmus reactions to caloric stimuli in children without ENG Technical errors would also be the most likely explanation of the remarkable observations by Koenigsberger et al (10) of a child with complete absence of vestibular reactions to caloric stimuli but with a normal rotation test Since both the caloric and rotation tests stimulate the lateral ampullae the results of these tests must agree

To further support his theory of a labyrinthine origin to paroxysmal torticollis Snyder (14) reports reduced hearing in 4 cases As he gives no information of the severity or type of hearing impairment apart from saying that there were no signs of otitis media it is impossible to assess the validity of these findings

In our series consanguinity between the parents was not found In one family (case 1) relatives with a history consistent with paroxysmal torticollis were found In the cases hitherto reported with paroxysmal torticollis where the sex is stated 13 out of 17 children have been females This tendency of female preponderance and the possible occurrence of similarly affected cases in the family might indicate that genetic causative factors might be working

However paroxysmal torticollis in infancy remains a puzzling periodic condition with many unsolved questions The site of dysfunction is probably the brain stem or the cerebellum The paroxysmal character could be explained by vascular or metabolic disturbances

It is important to recognize the condition because the parents can be reassured that the prognosis is good

ACKNOWLEDGEMENT

The authors wish to thank Mrs Kajsa Silwe for the excellent inkdrawing

REFERENCES

1 Brasser L S Benign paroxysmal vertigo of childhood *Brain 87* 141 1964
2 Casteels Van Daele M Paroxysmal torticollis in infancy *Am J Dis Child 120* 88 1970
3 Chutorian A M Benign paroxysmal torticollis tortipelvis and retrocollis in infancy *American Academy of Neurology* Twenty sixth Annual Meeting 1974
4 Crammer J L Periodic diseases Rhythm in medicine *Documenta Geigy* 1965
5 Ekbom K A Westerberg C E & Osterman P O Focal sensorymotor seizures of spinal origin *Lancet Jan 13* 67 1968
6 Fessard C Cerebral tumors in infancy *Am J Dis Child 115* 30 1968
7 Golden G S & French J H Basilar artery migraine in young children *Pediatrics 56* 7?? 1975
8 Gourley I M Paroxysmal torticollis in infancy *Can Med Assoc J 105* 504 1971
9 Henriksson N G Pfaltz C R Torok N & Rubin W A synopsis of the vestibular system An effort to standardize vestibular conceptions tests and their evaluation *Sando Monographs* 1972
10 Koenigsberger M R Chutorian A M Gold A P & Schvey M S Benign paroxysmal vertigo in childhood *Neurology (Minneap) 20* 1108 1970
11 Kyllerman M & Steen G Intermittently progressive dyskinetic syndrome in glutaric aciduria *Neuropaediatrie 8* 397 1977
12 Nilsson S E & Floderus S Nine cases of hereditary and nonhereditary periodic diseases *Acta Med Scand 175* 341 1964
13 Sanner G Bergstrom B & Kadesjo B Benign paroxysmal infantile torticollis *18 th Scandinavian Pediatric Congress* Aarhus 1976
14 Snyder C H Paroxysmal torticollis in infancy *Am J Dis Child 117* 458 1969
15 Sutcliffe J Torsion spasm and abnormal postures in children with hiatus hernia Sandifer s syndrome *P og Pediatr Rad ol* ? 190 1969
16 Tassinari C A & Fine R D Paroxysmal choreoathetosis *Proc Aust Assoc Neurol 6* 71 1969

Submitted June 10 1978
Accepted Aug 26 1978

(G S) Department of Paediatrics
Central Hospital
S-651 85 Karlstad
Sweden

drug could not prevent further attacks in our cases

Casteels Van Daele (2) stressed that paroxysmal torticollis could be a dystonic side reaction of phenothiazine therapy However this type of dystonia also involves the face and mouth, which distinguishes it from the condition of paroxysmal torticollis

A dystonic headposture also has been reported in association with hiatus hernia (15) In paroxysmal torticollis roentgen studies of the intestine have shown normal results (3) Furthermore this dystonic reaction is mostly seen at meals which was not the case in our patients A dystonic condition with a paroxysmal tendency has recently been described as an organic acid disorder (11) Therefore an urine sample was collected during the day of an attack in case 2 but when analyzed no abnormal organic acids could be found

The above mentioned differential diagnoses might be considered in certain cases However they do not agree with the regular periodicity of paroxysmal torticollis which seems to be a cardinal feature In 3 of our cases the free interval between attacks was about 14 days and in one case 1 month Mostly the parents could tell almost exactly when the next attack was to appear This is very striking and difficult to explain

In other diseases with regular periodicity the intervals often have been 7 14 or further multiples of 7 days (4) A similar pattern is seen in our series too Periodic diseases of this type are usually inherited (12)

The main feature of paroxysmal torticollis is the head tilting seen in all cases during infancy The fact that the abnormal posture is altered in different positions (case 2) may suggest that it is influenced by postural reflex systems Nevertheless it is quite clear that the clinical features of paroxysmal torticollis as they appear in infancy are influenced by the degree of maturation of the central nervous system as the condition alters character with increasing age This is exemplified by our case 2 where the attacks at 2 years of age more and more were characterized by paroxysmal ataxia with intention tremor and unsteady broadbased gait

The ataxic condition seen in case 2 rather points to the possibility of a paroxysmal dysfunction of the cerebellum or the vestibulocerebellar connections Theoretically a paroxysmal insufficiency of the basilar artery supplying these areas could produce this picture which is not unlike basilar artery migraine (7) However in migraine the attacks are shorter than those of paroxysmal torticollis usually are Furthermore in our series no child has got a paroxysmal headache and no close relatives are affected by migraine

Snyder (16) compared the torticollis to Meniere s syndrome in the adult and also suggested that it was identical with the syndrome of benign paroxysmal vertigo in childhood reported by Basser (1) Although Meniere s disease may occur in children there are no indications of a peripheral vestibular disorder as the cause of paroxysmal torticollis Ataxia which Snyder mentions as a proof of vestibular disorder is *not* a vestibular symptom Nine of Snyder s 12 cases and the case reported by Gourley (8) were said to have negative caloric reactions bilaterally To elicit a perception of dysequilibrium from the peripheral vestibular system there must be an imbalance between impulses from the right and left side (9) Loss of both labyrinths causes some balance problems but no vertigo In fact one method of treatment in cases of bilateral Meniere s disease is streptomycin in vestibulotoxic doses to eliminate the vestibular sensory epithelia in both ears

The same objection regarding the interpretation of the vestibular tests applies to the reports of Basser (1) and Koenigsberger et al (10) on paroxysmal vertigo in children Basser states that the predominant finding is moderate to complete canal paresis which may be unilateral or bilateral However in the 8 cases who were said to have bilateral canal paresis it was more pronounced on one side than the other It therefore seems probable

Acta Pædiatr Scand 68 225–227 1979

MYOTONIA CONGENITA (THOMSEN S DISEASE)

Early Diagnosis in Infancy

S HAREL L A CHUI and Y SHAPIRA

From the Neuromuscular Unit Department of Neurology Los Angeles County University of Southern California Medical Center Los Angeles California USA

ABSTRACT Harel S Chui A L and Shapira Y (Neuromuscular Unit Department of Neurology Los Angeles County University of Southern California Medical Center Los Angeles California) Myotonia congenita (Thomsen s disease) Acta Paediatr Scand 68 225 1979.—A family with myotonia congenita (Thomsen s disease) is reported in which the father and his two offspring are affected The course was characterized by the early onset of clinical manifestations in both the father and his two children In one child a clinical and electrical diagnosis of the disease was made as early as two weeks of age Early manifestations were breathing difficulty and eye closure myotonia The importance of early recognition of the disorder is emphasized

KEY WORDS Myotonia Thomsen s disease congenital stridor

Myotonia congenita (Thomsen s disease) is considered to be a relatively benign disorder characterized clinically by diffuse hypertrophy of voluntary muscles with widespread myotonia usually accentuated by rest and cold and relieved by exercise The usual mode of inheritance is autosomal dominant but autosomal recessive inheritance has also been reported (1 5 9 10) Symptoms are generally not apparent until childhood or adolescence although early symptoms of feeding difficulty and peculiar cry have been described (2 12) early diagnosis is uncommon and attention to such symptoms may lead to more frequent diagnosis in very young children In this paper we report a father and his two offspring who presented with unusual transient respiratory stridor as early symptoms of myotonia We believe that this is one of the earliest confirmed diagnoses of myotonia congenita reported

REPORT OF CASES

Case I (D J) a 8 year-old Caucasian male was referred for evaluation of his myotonic condition His chief complaint was that of occasional episodes of muscle stiffness and cramp involving the fa e and extremities His illness dated back to the neonatal period at which time he had several episodes of respiratory diffi ulty w h cyanosis and syncope which were attributed to mechanical airway obstruction At 8 months he developed difficulty in relaxing periorbital and masticatory muscles Later these difficulties progressed to affect both upper and lower extremities At two years the diagnosis of myotonia congenita was first made The patient claimed to be absolutely symptom free between episodes of myotonia His muscle symptoms occurred only immediately prior to or subsequent to febrile illnesses Only at these times does he describe difficulty opening his eyes and fists chewing solid foods as well as cramping pain in his arms and legs He had no history of muscle weakness or intermittent attacks of paralysis

Physical examination revealed a well developed extremely athletic looking man Pertinent neurological findings were confined to the musculature He showed generalized muscular hypertrophy most pronounced in the thenar and hypothenar eminences bilaterally Muscle strength and deep tendon reflexes in all extremities were normal He had percussion myotonia of the thenar eminences but no myotonia of the tongue could be elicited Active myotonia characterized by slow opening of the clenched fist and difficulty opening his eyes in forced closure of eyelids was observed *In some instances the myotonia became progressively more severe with repeated movements (paradoxical myotonia) and on other occasions the symptoms were partially relieved by exercise This observed pattern was consistent with the patient s own past experience Serum electrolytes during episodes of myotonia and at other times were within normal limits Ele tromyographic examination of the right thenar eminence muscle disclosed typical myotoni discharge*

Supported in part by grants from the Muscular Dystrophy Association of America

youngest patient this was easily done at the age of two weeks

Thus when confronted with a normal appearing and developing neonate with episodic feeding difficulty respiratory distress peculiar cry and eye closure myotonia the diagnosis of myotonia congenita should be considered even with an apparently negative family history since autosomal recessive inheritance is not uncommon (1 5 6 9 11) A brief EMG can then confirm the diagnosis

We feel that early recognition of the disease is imperative for differentiation from other life threatening conditions appropriate genetic counselling reassurance of the parents as to the benign course of the disease and possible early symptomatic relief with medication such as diphenylhydantoin (8)

REFERENCES

1 Becker P E *Proc 3 d National Cong ess Human Genetics* Chicago 1966

2 Bell D B & Smith D W Myotonic dystrophy in the neonate *J Pediatrics 81* 83 1972

3 Bell J Dystrophia myotonica and allied diseases *The Treasury Human Inheritance* London *4* 343 1947

4 Dodge P R Gamstorp I & Byers R K Myotonic dystrophy in infancy and childhood *Pediatrics* 35 3 1965

5 Harper P S & Dyken P R Early onset dystrophia myotonica *Lancet II* 53 1972

6 Kirby J F & Kraft G H Electromyographic studies in myotonia congenita *Arch Phys Med Rehabil 54* 47 1973

7 Klein D La dystrophie myotonique (Steinert) et la myotonie congenitale (Thomsen) en Suisse *J Genet Hum 7* Suppl P 1958

8 Munsat T L Therapy of myotonia A double blind evaluation of diphenylhydantoin procainamide and placebo *Neurology 17* 359 1967

9 Penders C A & Delwaide P J Formes cliniques de la myotonie congenitale none dystrophique *Acta Neurol Belg 72* 366 1972

10 Swaiman K F & Wright F S *Neuromuscular diseases of infancy and childhood* Charles C Thomas Springfield Ill 1970 p 197

11 Thomasen E Myotonia Thomsen s Disease Paramyotonia and Dystrophia Myotonia *Op Ex Domo Biol Hered Hum* Copenhagen E Munksgaard 1948 vol 43

12 Walton J N *Disorders of voluntary muscle* Churchill Livingstone London 1974 p 479

Submitted Jan 19 1978
Accepted Aug 7 1978

(S H) Pediatric Department A
Hadassah Hospital
Balfour Street 8
Tel Aviv
Israel

Case 2 (J D) the first child of Case 1 was first evaluated at the age of two months The child s perinatal history was unremarkable Early developmental milestones were normal He was a very alert and socially responsive baby who began smiling at the age of six weeks Because of the father s disease the parents had been watching the baby closely and reported that when the baby cried he had transient episodes of difficulty in opening his eyes the eyelids remaining closed for a few seconds They also mentioned that on several occasions the baby had noisy *inspiratory type breathing* (also described as losing his breath) for very short periods of time During these episodes no change in skin color was noted No feeding difficulty was reported

On examination the baby was a bright alert strong normally moving two month old No dysplastic features were seen Occasionally and especially when crying he *had short episodes of stridor accompanied by intercostal* and subcostal retractions His eyelids went into spasm and became difficult to open after crying The cranial nerves were otherwise intact Motor system examination revealed normally developed musculature with normal strength and tone Deep tendon reflexes were normal No myotonic response to muscle percussion could be elicited Sensory examination and primitive reflexes were normal Electromyographic examination using concentric needle electrodes was performed in his right deltoid first dorsal interosseous quadriceps anterior tibialis and gastrocnemius muscles Shortly after the insertion of the exploring needle electrode high frequency waxing and waning positive potentials identified as myotonic discharges were seen constantly in all muscles tested The motor unit profile on minimum and maximum contraction was normal

The baby was seen in follow up at two years of age His psychomotor development was normal He still had episodes of breathing difficulty ranging in frequency from several times per day to one or two times per week *While crying he continued to have difficulty with re*laxation and opening of his eyes

Case 3 (G J) the second child of D J was first evaluated at the age of two weeks She was born after an uncomplicated pregnancy and delivery Her mother observed that the baby had a peculiar cry with noisy inspiratory breathing similar to her older brother but there were *no eyelid or generalized myotonic symptoms* On examination the baby showed a normal developmental pattern There was no evidence of percussion or grip myotonia When crying she demonstrated short episodes of inspiratory stridor without cyanosis *All primi*tive reflexes were present

A brief electromyographic examination was performed on her *right first* dorsal interosseous anterior tibialis and gastrocnemius muscles Typical myotonic discharges were observed in all muscles tested The motor unit parameters were normal

DISCUSSION

The clinical course of the disease was characterized by the early onset of symptomatology in all three cases All experienced breathing difficulties in the neonatal period The appearance of eye closure myotonia was somewhat delayed in the father until about eight months of age Although Thomsen s disease is *known to be present from birth* symptoms are rarely apparent until late infancy or childhood in most reported cases and in our personal experience Affected infants have occasionally been described with symptoms such as *strangled cry* or *feeding difficulties* (2 3) Although pulmonary disability with emphysema and so called myotonic asthma has been described in older patients (17) neonatal respiratory symptoms have not been reported to our knowledge Despite some similarity the differentiation of Thomsen disease from myotonic dystrophy in the neonate is possible *In the latter disease* the clinical picture is much more severe and may include profound hypotonia weakness facial paresis cranial dysmorphism decreased muscle mass poor feeding respiratory distress *talipes equinovarus* cryptorchidism and hypomineralized ribs and long bones (2 4 7)

Although electrical myotonia was present in all three cases the children showed no mechanically induced myotonia and in the father the myotonia was present only in the thenar eminence The inconsistent response of the father s myotonia to exercise differs somewhat from the usual improvement of myotonia after exercise Myotonia is usually accentuated by cold but was induced in the father only by fever in the course of *febrile illnesses*

Myotonia congenita can present as a polymorphous picture with varying clinical manifestations (5 9) Contrary to the clinical picture the electromyographic findings in myotonia congenita are very consistent Many are still for obvious reasons reluctant to perform electromyographic studies on young *infants but in thomsen s disease* myotonia is widespread and the myotonic response to electrical stimulation is so typical that one can perform a brief EMG on only *few muscles* for confirmation of the diagnosis In our

Acta Pædiatr Scand 68 229–237 1979

EARLY DETECTION OF PRESCHOOL HEALTH PROBLEMS—ROLE OF PERINATAL RISK FACTORS

LENNART KÖHLER NILS W SVENNINGSEN and BERTIL LINDQUIST

From the Department of Paediatrics University Hospital Lund Sweden

ABSTRACT Kohler L Svenningsen N W and Lindquist B (Department of Paediatrics University Hospital Lund Sweden) Early detection of preschool health problems—role of perinatal risk factors Acta Paediatr Scand 68 2?9 1979 —To evaluate a perinatal risk grouping system 126? 4-year-old children went through a comprehensive health examination A total of 41 5% of the newborns were included in the wide criteria of risk which were more common among boys and among children of the youngest and oldest mothers Among the 4-year old the frequency of significant physical health problem was 15 8% including 10 1% visual disturbances and 2% neurological disorders In some combinations of riskgroups and later health problems there were statistically significant correlations e g regarding prematurity and cerebral irritation vs cerebral palsy but not sufficient to serve its purpose as a screening instrument Even the accumulation of especially serious events in the perinatal period gave no clue to later neurological disorders The addition of low socio-economic status as a perinatal risk did not influence the outcome either The reasons for the weak correlation between perinatal risk factors and later outcome of health disorders and handicaps are discussed and it is concluded that to detect children with health problems there seems to be no acceptable alternative to a comprehensive health surveillance as part of a general health service programme of all children including clinical examinations and screening procedures by well trained personnel

KEY WORDS Children at risk preschool health problems neurological disorders cerebral palsy visual disturbances behaviour problems

Preventive paediatrics in Sweden is provided by the Child Health Centres According to regulations these centres should perform health examinations of the children s physical mental and social development from birth until they start school at the age of seven years The main purpose of this close health surveillance is to ensure an early recognition of disabling conditions and handicapping disorders By the introduction of a special and elaborate health examination of all 4 year old children a still more efficient tool in this early detection programme was created leading to the finding of a reasonable number of previously unknown important deviations especially by screening examinations of vision hearing bacteriuria and behaviour (12–17)

It was concluded that a thorough paediatric examination at this age revealed rather few children with unknown functionally important health problems especially when compared with the time consuming and expensive methods of examination and that other ways of early detection of children with physical health problems should be investigated (17)

The interest was then directed towards the concept of children at risk i e to select by some criteria at a very early age children specially liable to later develop health problems Usually such criteria are based on gestational and neonatal events (29 32) but other factors should also be considered e g social and environmental ones (22 28)

The purpose of the present study was to evaluate such a risk grouping system of newborns by examining the children at the age of 4 years

MATERIAL

In two consecutive years ?? 4 children belonging to the community of Lund were born in the maternity ward University Hospital Lund Four years later 1 ?8 of these children were still resident in Lund and 1 ?6? of them were given a comprehensive health examination

Table 2 *Classification of health problems*

Groups ? and 3 are termed significant health problems (see text)

Method of examination	Group 0 Healthy child	Group 1 Slight deviation without importance	Group ? Moderate deviation Treatment indicated	Group 3 Definitely handicapping disorder
General medical examination or anamnestical data		Flat feet phimosis slight eczema	Minimal brain dysfunction retentio testis urinary tract infection	Cerebral palsy organic heart disease severe epilepsy juvenile rheumatic arthritis
Vision examination		Myopia ≤ −1 0 D Hyperopia +2 ?5–3 75 D Astigmatism ≤ −1 5 d ×0° or ≤ −1 0 D×90°	Myopia > −1 0 D Hyperopia ≥ +4 D Astigmatism ≥ − 0 D×0° or ≥ −1 5 D×90° Anisometropia ≥? 0 D	Amblyopia strabismus
Auditory examination		Mild otosalpingitis cured by simple otological measures and few visits to the physician	Protracted otosalpingitis requiring more intense therapy (adenoidectomy drainage tubes) Moderate sensorineural hearing impairments with need for follow up and control	Severe impairment requiring hearing aid or special education (training)

oldest mothers and among single mothers neonatal reasons were rarest among the youngest mothers

The findings of important health problems among the children at the age of 4 years are shown in Table 5 The total percentage of significant physical health problems was 15 8% which is the same as found in the larger health examination performed some years previously 16 6% ($p>0$ 05) (15)

There was little difference between subgroups neurological disorders in this study 2 0% versus 2 8% previously visual disorders 10 1% vs 9 0% auditory disorders 2 5% vs 1 7% ($p>0$ 05) (13–15)

The somatic health problems are specified in Table 6 The prevalence of different neurological disorders was also similar between surveys minimal brain dysfunction (hyperactivity and distractibility combined with general clumsiness but without additional neurological signs) 0 9% vs 1 1% cerebral palsy 0 4% vs 0 4% psycho motor retardation 0 6% vs 0 3% and epilepsy 0 1% vs 0 1% ($p>0$ 05)

Considerable behaviour problems were noted by the parents less frequently in this study 7 4% vs 10 2% in the previous one ($p<0$ 01) (17)

Enuresis more than two nights a week was equally frequent 9 3% vs 8 7% ($p>0$ 05)

Table 7 shows the connection between risk factors and health problems at 4 years of age

It is evident from this table that rather few differences in health problems existed at the

Table 3 *Classification of the participants in a screening programme*

Screening results	Diagnosis: Diseased	Non diseased	Total
Positive	a	b	$a+b$
Negative	c	d	$c+d$
Total	$a+c$	$b+d$	$a+b+c+d$

Sensitivity = $\frac{a}{a+c}\times 100$

Specificity = $\frac{d}{b+d}\times 100$

Positive predictive value = $\frac{a}{a+b}\times 100$

Youden index = sensitivity + specificity − 100

A Youden index of 50% or less means a secreening test no better than random chance (5)

Table 1. *Newborn children at risk*

A. *Genetic reasons*

Family history of diseases in eyes, ears or nervous system

B. *Prenatal reasons* (disorders by the mother)

1. Endocrine disorders, e.g. diabetes, thyroid disturbances
2. Disorders in the circulatory system, e.g. heart, lungs or blood
3. Infectious diseases, e.g. rubella or other virus infections (in first 16 weeks of pregnancy), lues, toxoplasmosis, pyelitis, severe bacterial infection (meningitis, sepsis)
4. Complications of pregnancy, e.g. toxaemia, imminent abortion, repeated or large vaginal haemorrhages
5. Drugs in repeated doses, e.g. chemotherapy, antibiotics, cytostatics, hormones, hypnotics, X-ray other than chest X-ray, major surgery in early months of pregnancy
   Preventive drugs, such as vitamins and iron, given *lege artis*, are not registered
6. Psychiatric illness

C. *Perinatal reasons*

1. Prolonged or difficult labour, e.g. by instrument, breech presentation, primiparae >35 years of age, multiple birth, early rupture of membranes (more than 12 hours before partus), prolapse of umbilical cord
2. *Disorders* of the placenta, e.g. placenta praevia, abruption or placenta with many infarcts, calcifications or larger haemorrhages in the placenta. *Weight* of placenta >900 g and <300 g by relatively normal birth weight in relation to gestational age. Only one umbilical artery
3. Short gestation (<36 weeks) and/or low birth weight (<2 200 g) (i.e. prematurity in the present study)
4. Intra- or extrauterine asphyxia (slow, irregular foetal heart rate with or without meconium stained fluid. Apgar score 0–6 at 1, 5 or 10 min. Also *neonatal* distress during the following days (respiratory insufficiency with apnoea or hyaline membrane disease, neonatal hypoglycemia)

D. *Neonatal reasons*

1. Cerebral irritation, e.g. convulsions, hyperexcitability, hypertonia, other signs of pathological nervous findings
2. Low vitality, e.g. obvious feeding difficulties, weight loss >15%, floppy infant syndrome
3. Jaundice, leading to exchange therapy (2)
4. Malformations as listed by the National Board of Health and Welfare (11)
5. Dysplastic children, e.g. low set ears
6. Other manifest or suspected abnormalities, e.g. too wide or too narrow cranial sutures, strange smell, cry or look

METHODS

In the maternity ward, all newborn children were examined by an experienced neonatologist (NWS), on their first or second day of life according to a standardized scheme. As well as a physical examination and test of neonatal reflexes, the degree of maturity was also evaluated: assessment of external features, neurological deve... ment and head circumference (4). The physical and neu... logical examinations were repeated on the day of ... charge from the hospital, i.e. usually on day 5. Child... fulfilling the criteria of "risk-children" (Table I) w... listed and the record was kept separately. Children ... needed immediate transfer to the neonatal intensive c... unit were included in the risk register after discha... from the hospital.

The routine examinations and health surveillance wi... the Child Health Services were performed as usual; ... children needing medical attention were taken care ... according to prevailing routines.

At the age of 4 years the children passed a comp... hensive health examination including a thorough phys... check up by an experienced paediatrician (L. K.) ... screening of vision, hearing, bacteriuria and behaviou... problems. Children with health problems were referred ... further evaluation by respective specialists at the Univ... sity Hospital of Lund. Details of these procedures ha... been published previously (16). The examination w... supplemented with questionnaires to the parents regardi... the child's development, previous and present health pro... lems, and the family's social and educational standard ... 3 graded socio-economic grouping system widely used ... Sweden was also employed (3). This system is bas... on paternal occupation, group I representing the high... group. The records of children already under professio... care were checked but otherwise, the information f... the parents was unconfirmed. The existing records ... those 20 children who failed to appear at the health e... amination, were checked at the Department of Paediatric... which is the main centre caring for sick and handicapp... children in this area. Diseases and handicaps of these chi... dren were included in the final calculation of morbidi... and perinatal risks.

After the professional evaluation of the health problem... an attempt was made to classify them according to the... severity or importance for the child's wellbeing (Table ... Deviations in groups 2 and 3 were regarded as functionall... important health problems and were termed significan... while the slight deviations were termed insignificar... (16).

Chi square analyses were used, with Yates' correctio... where applicable.

In evaluating the screening procedures, definition... according to Table 3 were used (5).

RESULTS

The criteria for risk grouping were very wide and a total of 41.5% of the newborns were included with a small preponderance of boys, 46.9% vs. 36.7% for girls. The distribution of various risk groups in relation to the mothers' age, marital status and socio economic standard is shown in Table 4. Perinatal reasons were more common among the youngest and the

r	II	III	Statistical differences risk–no risk
)	0	100	
	31 7	31 2	
4 4	?	33 3	
3 3	3 3	33 3	
4 8	?9	6 0	
3 7	39 5	36 8	
?	44 4	33 3	
0	50 0	50 0	
9 7	3 8	37 5	
9 8	31 8	38 4	
0 6	30 6	38 9	
6 4	28 ?	56 4	
C 8	3 8	34 3	
	35 6	3 ?	
1	43 9	39 0	
4 6	33 3	3_ 1	
0 0	10 0	0 0	
5 0	43 8	31	
1 1		66 7	
4 7	38 9	6 4	
6	33 4	34 0	
6	?9 8	3 6	

(Column group heading: ocio-economic group)

DISCUSSION

There is a general agreement among paediatricians that early detection of most handicapping disorders makes the treatment easier and the results better Since many of the most serious and disabling conditions are retrospectively connected with adverse events during pregnancy and neonatal period (9 19 ?0) the concept of risk groups theoretically seems to be an attractive idea

However in spite of considerable enthusiasm for the idea especially in Great Britain (29) the results of most studies have been disappointing Not only did these risk groups come to include merely a part of the children with subsequent handicaps (6 10 21 26) but they also included a lot of children who grew up to be perfectly normal (23 31) Furthermore mild perinatal complications could have extensive effects and high risk conditions may survive without signs of damage (25) Besides the procedure of labelling and following up of certain children may influence their development and affect the parent–child relationship (25)

It is evident that there are great methodological difficulties in studies of this kind the sampling of patients studied the selection and assessment of risk criteria and the evaluation of the follow up result Problems of this kind are of course bound to happen especially when a great number of midwives and doctors with differing backgrounds training and equipment examine and assess the children Therefore it was thought that a strict scheme and standardized examinations performed by the same welltrained staff on a group of children selected only by their place of residence could make it possible to overcome some of these problems Since the children were followed by the routine health examinations in the ordinary health surveillance system without access to the risk grouping the effect of labelling was thought to be minimal if any

It is evident that too many children were contained in the risk register but the purpose was to include every known or suspected risk factor in a way that made it possible to select factors with the greatest *impact on the children s subsequent health*

At 4 years of age the children were very

Table 6 *Specification of significant somatic health problems among 1282 4 year old children*

	n	%
Neurological disorders	6	2 0
Minimal brain dysfunction	17	0 9
Cerebral palsy	5	0 4
Psycho-motor retardation	7	0 6
Epilepsy		0 1
Other somatic disorders	15	1 1
Gigantism	1	0 1
Organic heart disease	4	0 3
Diabetes	1	0 1
Urinary tract infection	1	0 1
Haemophilia A	1	0 1
Retentio testis	1	0 1
Congenital hip dislocation	5	0 4
Juvenile rheumatic arthritis	1	0 1
Toal	41	3 2

Table 4 *Distribution of 1282 risk grouped children on the mothers age, marital status and economic group (percentages)*

	Mothers							
	Age in years				Statistical difference risk–no risk	Marital status		Statisti differe risk–n
Risk groups	–19	20–29	30–39	40–		Married	Single	
A Genetic reasons (*n*=1)	0	100	0	0		100	0	
B Prenatal reasons (*n*=197)	6.1	67.6	24.9	1.5		89.4	10.6	
1 Endocrine (*n*=9)	11.1	77.8	11.1	0		88.9	11.1	
2 Circulatory (*n*=30)	6.7	70.0	23.3	0		96.7	3.3	
3 Infections (*n*=95)	5.3	70.5	23.2	1.1		90.6	9.4	
4 Pregn complic (*n*=75)	5.3	60.0	32.0	2.7		90.7	9.3	
5 Drugs (*n*=9)	22.2	66.7	11.1	0		66.7	33.3	
6 Psychiatric (*n*=2)	0	100	0	0		50.0	50.0	
C Perinatal reasons (*n*=258)	9.4	64.4	23.1	3.1		84.4	15.6	*
1 Difficult labour (*n*=151)	7.9	61.0	25.8	5.3	*	90.1	9.9	
2 Placenta disorders (*n*=72)	8.3	63.9	26.4	1.4		81.9	18.1	
3 Prematurity (*n*=48)	7.7	74.3	7.7	10.3		82.1	17.9	
4 Asphyxia (*n*=68)	16.4	56.8	22.4	4.5		79.1	20.9	
D Neonatal reasons (*n*=210)	1.9	74.5	22.6	1.0		92.3	7.7	
1 Cerebral irritation (*n*=42)	4.9	80.5	14.7	0		87.8	12.2	
2 Low vitality (*n*=82)	1.2	69.1	28.4	1.2		90.1	9.9	
3 Jaundice (*n*=10)	0	60.0	40.0	0		100.0	0	
4 Malformations (*n*=16)	6.3	68.8	25.0	0		100.0	0	
5 Dysplasia (*n*=9)	0	66.7	33.3	0		100.0	0	
6 Other (*n*=72)	1.4	80.6	16.7	1.4		91.7	8.3	
Any risk (*n*=532)	6.1	68.0	23.9	2.0		88.1	11.9	
No risk (*n*=750)	6.7	67.9	24.0	1.4		91.5	9.9	

age of 4 years between children considered to be at risk and children not considered to be at risk in the newborn period. Although the differences in some risk groups are statisti significant, they are in no instance high en to serve as prognostic signs, i.e. the sensit as well as the positive predictive value o risk grouping as a screening method are Thus, e.g. in using prematurity as a scre test for later outcome of cerebral palsy sensitivity will be 60%, the specificity 9 the Youden index 57%, and the positive dictive value 7.5%. The combination of factors (24) shown in Table 8 did not ch the outcome of the screening. The corresp ing figures for prematurity combined with c bral irritation will be 40%, 99.8%, 40% 39.8% respectively. Even the "foetal dep tion of supply" (7, 27) alone (number 4 Table 8) or in combination with neonatal s (number 5*a* in Table 8) did not select neurological disorders.

Table 5 *Health problems among 1282 four year old children*

	n	%
Significant somatic health problems	41	3.2
Neurological disorders	26	2.0
Other somatic disorders	15	1.2
Significant visual disorders	130	10.1
Strabismus	42	3.2
Refractive errors only	88	6.9
Significant auditory disorders	32	2.5
Sensorineural	3	0.2
Conductive	29	2.3
Sum significant physical health problems	203	15.8
Problems with upbringing and behaviour		
Considerable behaviour problems	93	7.4
Enuretic > two nights a week	117	9.3
Very late speech development	12	1.0

The addition of low socio economic st as a perinatal risk group did not influence outcome either (Table 8).

...cio-economic group			Statistical differences
...	II	III	risk–no risk
	0	100	
	31 7	31	
4		33 3	
3	3 3	33 3	
8	29	6 0	
7	39 5	36 8	
	44 4	33 3	
	50 0	50 0	
7	3 8	37 5	
8	31 8	38 4	
6	30 6	38 9	
4	8 2	56 4	
8	3 8	34 3	
	35 6	32	
1	43 9	39 0	
6	33 3	32 1	
0	10 0	20 0	
0	43 8	31	
1	22	66 7	
7	38 9	6 4	
6	33 4	34 0	
6	9 8	3 6	

DISCUSSION

There is a general agreement among paediatricians that early detection of most handicapping disorders makes the treatment easier and the results better Since many of the most serious and disabling conditions are retrospectively connected with adverse events during pregnancy and neonatal period (9 19 20) the concept of risk groups theoretically seems to be an attractive idea

However in spite of considerable enthusiasm for the idea especially in Great Britain (29) the results of most studies have been disappointing Not only did these risk groups come to include merely a part of the children with subsequent handicaps (6 10 21 26) but they also included a lot of children who grew up to be perfectly normal (23 31) Furthermore mild perinatal complications could have extensive effects and high risk conditions may survive without signs of damage (25) Besides the procedure of labelling and following up of certain children may influence their development and affect the parent–child relationship (25)

It is evident that there are great methodological difficulties in studies of this kind the sampling of patients studied the selection and assessment of risk criteria and the evaluation of the follow up result Problems of this kind are of course bound to happen especially when a great number of midwives and doctors with differing backgrounds training and equipment examine and assess the children Therefore it was thought that a strict scheme and standardized examinations performed by the same welltrained staff on a group of children selected only by their place of residence could make it possible to overcome some of these problems Since the children were followed by the routine health examinations in the ordinary health surveillance system without access to the risk grouping the effect of labelling was thought to be minimal if any

It is evident that too many children were contained in the risk register but the purpose was to include every known or suspected risk factor in a way that made it possible to select factors with the greatest impact on the children s subsequent health

At 4 years of age the children were very

Table 6 *Specification of significant somatic health problems among 1282 4 year old children*

	n		%	
Neurological disorders	26		2 0	
Minimal brain dysfunction		12		0 9
Cerebral palsy		5		0 4
Psycho-motor retardation		7		0 6
Epilepsy		2		0 1
Other somatic disorders	15		1 1	
Gigantism		1		0 1
Organic heart disease		4		0 3
Diabetes		1		0 1
Urinary tract infection		1		0 1
Haemophilia A		1		0 1
Retentio testis		5		0 4
Congenital hip dislocation		1		0 1
Juvenile rheumatic arthritis		1		0 1
Total	41		3 2	

tology has been very rapid new electronic equipment for surveillance during pregnancy and labour has been produced new indications for instrumental deliveries have been applied wider criteria and more efficient use of assisted breathing of the newborn have come into practice Also other definitions and terms are prevailing A study starting to day would of course include small-for-date children and use a 24 hours limit for early rupture of membranes instead of 12 hours and set the border of low birth weight of 2 500 g instead of 2 200 g This means that the risk factors used in this study to predict the outcome of to days 4 year olds do not reflect the present situation of the newborns However there is no other way of assessing the importance of perinatal events than to examine the children many years later Maybe even 4 years is too short a time some of the health problems will not appear until greater demands on intellectual and perceptual functions are made on the children in school A new study including a foetal monitoring of all the subjects is now going on and the children are to be followed to school age

For the time being however there seems to be no acceptable alternative to a comprehensive health surveillance as part of a general health service programme of all children including clinical examinations and screening procedures by well trained personnel For the parents obstetricians and neonatologists it may be of some satisfaction that children at risk according to traditional criteria do not develop more handicaps than other children

ACKNOWLEDGEMENT

We are indebted to the Malmohus County Council for the support of this study

REFERENCES

1 Alberman E D & Goldstein H The at risk register A statistical evaluation *Br J Prev Soc Med 24* 129 1970

2 Avery G B Neonatology *Pathophysiology and management of the newborn* J P Lippincott Co Philadelphia 1975 pp 347–350

3 *Elections for the Riksdag* Central Bureau of Statistics Stockholm 1953

4 Finnstrom O Studies on maturity in newborn infants VI Comparison between different methods for maturity estimation *Acta Paediatr Scand 61* 1972

5 Frankenburg W K Pediatric screening *In* Schulman (ed) *Advances in pediatrics* vol 20 Year Book Medical Publishers Chicago 1973

6 Hamilton F M W The early detection of handicaps in children *Med Offr 120* 167 1968

7 Hagberg G Hagberg B & Olow I The changing panorama of cerebral palsy in Sweden 1954-19 The importance of foetal deprivation of supply *Acta Paediatr Scand 65* 403 1976

8 Hofsten E Social classes in Swedish statistics production *Statistisk Tidskrift 5* 368 1967

9 Illingworth R S The diagnosis of cerebral palsy in the first year of life *Develop Med Child Neur 8* 178 1966

10 Knox E G & Mahon D F Evaluation of infant at risk register *Arch Dis Child 45* 634 1970

11 Kallén B & Winberg J A Swedish register of congenital malformations *Pediatrics 41* 765 1968

12 Kohler L Fritz H & Schersten B Bacteriuria in 4 year old children *Acta Paediatr Scand 61* 1972

13 Kohler L & Holst H E Auditory screening of four year-old children *Acta Paediatr Scand 61* 555 1972

14 Kohler L & Stigmar G Vision screening of four year old children *Acta Paediatr Scand 62* 17 1973

15 Kohler L Physical health of four year-old children *Acta Paediatr Scand 62* 181 1973

16 Kohler L Health control of four year-old children An epidemiological study of child health *Acta Paediatr Scand* Suppl 235 1973

17 Kohler E M & Kohler L Health and behaviour of four year-old children *Acta Paediatr Scand 64* 25 1975

18 Kohler L Physical health of 7 year-old children An epidemiological study of school entrants and a comparison with preschool health *Acta Paediatr Scand 66* 297 1977

19 Lilienfeld A M & Pasamanick B The association of maternal and fetal factors with the development of cerebral palsy and epilepsy *Am J Obstet Gynecol 70* 93 1955

20 Little J W Lecture VIII in Course of lectures on the deformities of the human frame *Lancet* p 319 1843

21 Mitchell R G Changing concept of risk *Develop Med Child Neurol 17* 277 1975

22 Neligan G A Kolvin I Scott D McI & Garside R F *Born too soon or born too small A follow up study to seven years of age* Clinics in Developmental Medicine No 61 Heinemann London 1976

23 Oppe T E Risk registers for babies *Develop Med Child Neurol 9* 13 1967

24 Parmelee A H & Haber A Who is the Risk infant? *Clinical Obstet Gynecol 16* 376 1973
25 Prechtl H F R Chairman s conclusion *In* Z K Stembera K Polacek & V Sabata (eds) *Perinatal medicine* Georg Thieme Stuttgart 1975
26 Rogers M G H The early recognition of handicapping disorders in childhood *Develop Med Child Neurol 13* 88 1971
27 Sabel K G Olegård R & Victorin L Remaining sequelae with modern perinatal care *Pediatrics 57* 652 1976
28 Sameroff A J Early influences on development—fact or fancy? *Merrill Palmer Quarterly of Behaviour and Development 21* 267 1975
29 Sheridan M Infants at risk of handicapping conditions *Mon Bull Min Health 21* 238 196
30 Stratton P M Criteria for assessing the influence of obstetric circumstances on later development *In* T Chard & M Richards (eds) *Benefits and hazards of the new obstetrics* Clinics in Developmental Medicine No 64 William Heinemann London 1977
31 Thomson A J Searle M & Russell G Quality of survival after severe birth asfyxia *Arch Dis Child 52* 620 1977
32 Walker R G An assessment of the current status of the at risk register *Scottish Home and Health Department* 1967

Submitted April 11 1978
Accepted Sept 1 1978

(L K) Department of Paediatrics
University Hospital
S-2 185 Lund
Sweden

tology has been very rapid new electronic equipment for surveillance during pregnancy and labour has been produced new indications for instrumental deliveries have been applied wider criteria and more efficient use of assisted breathing of the newborn have come into practice Also other definitions and terms are prevailing A study starting today would of course include small-for-date children and use a 24 hours limit for early rupture of membranes instead of 12 hours and set the border of low birth weight of 2500 g instead of 2200 g This means that the risk factors used in this study to predict the outcome of today's 4 year olds do not reflect the present situation of the newborns However there is no other way of assessing the importance of perinatal events than to examine the children many years later Maybe even 4 years is too short a time some of the health problems will not appear until greater demands on intellectual and perceptual functions are made on the children in school A new study including a foetal monitoring of all the subjects is now going on and the children are to be followed to school age

For the time being however there seems to be no acceptable alternative to a comprehensive health surveillance as part of a general health service programme of all children including clinical examinations and screening procedures by well trained personnel For the parents obstetricians and neonatologists it may be of some satisfaction that children at risk according to traditional criteria do not develop more handicaps than other children

ACKNOWLEDGEMENT

We are indebted to the Malmöhus County Council for the support of this study

REFERENCES

1 Alberman E D & Goldstein H The at risk register A statistical evaluation *Br J Prev Soc Med* 24 129 1970

2 Avery G B *Neonatology Pathophysiology and management of the newborn* J P Lippincott Co Philadelphia 1975 pp 347–350

3 *Elections for the Riksdag* Central Bureau of Statistics Stockholm 1953

4 Finnström O Studies on maturity in newborn infants VI Comparison between different methods for maturity estimation *Acta Paediatr Scand* 61 33 1972

5 Frankenburg W K Pediatric screening *In* J Schulman (ed) *Advances in pediatrics* vol 20 Year Book Medical Publishers Chicago 1973

6 Hamilton F M W The early detection of handicap in children *Med Offr* 120 167 1968

7 Hagberg G Hagberg B & Olow I The changing panorama of cerebral palsy in Sweden 1954–1970 The importance of foetal deprivation of supply *Acta Paediatr Scand* 65 403 1976

8 Hofsten E Social classes in Swedish statistics production *Statistisk Tidskrift* 5 368 1967

9 Illingworth R S The diagnosis of cerebral palsy in the first year of life *Develop Med Child Neurol* 8 178 1966

10 Knox L G & Mahon D F Evaluation of infant at risk register *Arch Dis Child* 45 634 1970

11 Kallén B & Winberg J A Swedish register of congenital malformations *Pediatrics* 41 765 1968

12 Köhler L Fritz H & Scherstén B Bacteriuria in 4 year old children *Acta Paediatr Scand* 61 289 1972

13 Köhler L & Holst H E Auditory screening of four year-old children *Acta Paediatr Scand* 61 555 1972

14 Köhler L & Stigmar G Vision screening of four year old children *Acta Paediatr Scand* 62 17 1973

15 Köhler L Physical health of four year-old children *Acta Paediatr Scand* 62 181 1973

16 Köhler L Health control of four year-old children An epidemiological study of child health *Acta Paediatr Scand* Suppl 235 1973

17 Köhler E M & Köhler L Health and behaviour of four year old children *Acta Paediatr Scand* 64 275 1975

18 Köhler L Physical health of 7 year old children An epidemiological study of school entrants and a comparison with preschool health *Acta Paediatr Scand* 66 297 1977

19 Lilienfeld A M & Pasamanick B The association of maternal and fetal factors with the development of cerebral palsy and epilepsy *Am J Obstet Gynecol* 70 93 1955

20 Little J W Lecture VIII in Course of lectures on the deformities of the human frame *Lancet* p 319 1843

21 Mitchell R G Changing concept of risk *Develop Med Child Neurol* 17 277 1975

22 Neligan G A Kolvin I Scott D McI & Garside R F *Born too soon or born too small A follow up study to seven years of age* Clinics in Developmental Medicine No 61 Heinemann London 1976

23 Oppé T E Risk registers for babies *Develop Med Child Neurol* 9 13 1967

?4 Parmelee A H & Haber A Who is the Risk infant ? *Clinical Obstet Gynecol 16* 376 1973

?5 Prechtl H F R Chairman s conclusion *In* Z K Stembera K Polacek & V Sabata (eds) *Perinatal medicine* Georg Thieme Stuttgart 1975

?6 Rogers M G H The early recognition of handicapping disorders in childhood *Develop Med Child Neurol 13* 88 1971

7 Sabel K G Olegård R & Victorin L Remaining sequelae with modern perinatal care *Pediatrics 57* 65? 1976

8 Sameroff A J Early influences on development—fact or fancy? *Merrill Palmer Quarterly of Behaviour and Development ?1* 67 1975

?9 Sheridan M Infants at risk of handicapping conditions *Mon Bull Min Health 21* 238 196?

30 Stratton P M Criteria for assessing the influence of obstetric circumstances on later development *In* T Chard & M Richards (eds) *Benefits and hazards of the new obstetrics* Clinics in Developmental Medicine No 64 William Heinemann London 1977

31 Thomson A J Searle M & Russell G Quality of survival after severe birth asfyxia *Arch Dis Child 5?* 6?0 1977

3 Walker R G An assessment of the current status of the at risk register *Scottish Home and Health Department* 1967

Submitted April 11 1978
Accepted Sept 1 1978

(L K) Department of Paediatrics
University Hospital
S ?21 85 Lund
Sweden

tology has been very rapid new electronic equipment for surveillance during pregnancy and labour has been produced new indications for instrumental deliveries have been applied wider criteria and more efficient use of assisted breathing of the newborn have come into practice Also other definitions and terms are prevailing A study starting to day would of course include small for date children and use a 24 hours limit for early rupture of membranes instead of 12 hours and set the border of low birth weight of 2500 g instead of 2200 g This means that the risk factors used in this study to predict the outcome of to days 4 year olds do not reflect the present situation of the newborns However there is no other way of assessing the importance of perinatal events than to examine the children many years later Maybe even 4 years is too short a time some of the health problems will not appear until greater demands on intellectual and perceptual functions are made on the children in school A new study including a foetal monitoring of all the subjects is now going on and the children are to be followed to school age

For the time being however there seems to be no acceptable alternative to a comprehensive health surveillance as part of a general health service programme of all children including clinical examinations and screening procedures by well trained personnel For the parents obstetricians and neonatologists it may be of some satisfaction that children at risk according to traditional criteria do not develop more handicaps than other children

ACKNOWLEDGEMENT

We are indebted to the Malmohus County Council for the support of this study

REFERENCES

1 Alberman E D & Goldstein H The at risk register A statistical evaluation *Br J Prev Soc Med* 24 129 1970
2 Avery G B *Neonatology Pathophysiology and management of the newborn* J P Lippincott Co Philadelphia 1975 pp 347–350
3 *Elections for the Riksdag* Central Bureau of Statistics Stockholm 1953
4 Finnstrom O Studies on maturity in newborn infants VI Comparison between different methods for maturity estimation *Acta Paediatr Scand 61* 33 1972
5 Frankenburg W K Pediatric screening *In* J Schulman (ed) *Advances in pediatrics* vol 20 Year Book Medical Publishers Chicago 1973
6 Hamilton F M W The early detection of handicap in children *Med Offr 120* 167 1968
7 Hagberg G Hagberg B & Olow I The changing panorama of cerebral palsy in Sweden 1954-1970 The importance of foetal deprivation of supply *Acta Paediatr Scand 65* 403 1976
8 Hofsten E Social classes in Swedish statistics production *Statistisk Tidskrift 5* 368 1967
9 Illingworth R S The diagnosis of cerebral palsy in the first year of life *Develop Med Child Neurol 8* 178 1966
10 Knox E G & Mahon D F Evaluation of Infant at risk register *Arch Dis Child 45* 634 1970
11 Kallen B & Winberg J A Swedish register of congenital malformations *Pediatrics 41* 765 1968
12 Kohler L Fritz H & Schersten B Bacteriuria in 4 year-old children *Acta Paediatr Scand 61* 789 1972
13 Kohler L & Holst H E Auditory screening of four year-old children *Acta Paediatr Scand 61* 555 1972
14 Kohler L & Stigmar G Vision screening of four year old children *Acta Paediatr Scand 62* 17 1973
15 Kohler L Physical health of four year-old children *Acta Paediatr Scand 62* 181 1973
16 Kohler L Health control of four year old children An epidemiological study of child health *Acta Paediatr Scand* Suppl 235 1973
17 Kohler E M & Kohler L Health and behaviour of four year old children *Acta Paediatr Scand 64* 225 1975
18 Kohler L Physical health of 7 year-old children An epidemiological study of school entrants and a comparison with preschool health *Acta Paediatr Scand 66* 297 1977
19 Lilienfeld A M & Pasamanick B The association of maternal and fetal factors with the development of cerebral palsy and epilepsy *Am J Obstet Gynecol 70* 93 1955
20 Little J W Lecture VIII in Course of lectures on the deformities of the human frame *Lancet* p 319 1843
21 Mitchell R G Changing concept of risk *Develop Med Child Neurol 17* 277 1975
22 Neligan G A Kolvin I Scott D McI & Garside R F *Born too soon or born too small A follow up study to seven years of age* Clinics in Developmental Medicine No 61 Heinemann London 1976
23 Oppé T E Risk registers for babies *Develop Med Child Neurol 9* 13 1967

Acta Pædiatr Scand 68 239–243 1979

PLASMA AND RED BLOOD CELL FOLATE IN BREASTFED INFANTS

J EK and E M MAGNUS

From the Department of Paediatrics Rikshospitalet and Kroghstotten Department of Oslo City Hospital Oslo Norway

ABSTRACT Ek J and Magnus E M (Department of Paediatrics Rikshospitalet and Kroghstotten Department of Oslo City Hospital Oslo Norway) Plasma and red blood cell folate in breastfed infants Acta Paediatr Scand 68 239 1979 —We have studied growth red blood cell status and folate concentrations in plasma and red cells in a group of 35 breastfed infants during the first year of life Folic acid supplementation was not given to the mothers during pregnancy or lactation and none of them developed megaloblastic anaemia The growth and red blood cell status of the infants were both normal At birth and throughout the period of observation the folate concentrations in plasma and red cells were significantly higher than in the adult reference material A positive correlation between plasma and red cell folate was demonstrated During the latter part of pregnancy and lactation the foetuses and infants seem to be protected against folate deficiency We regard the folate status of normal breastfed infants as optimal The optimal supply of the vitamin in artificial nutrition should be the amount of folate necessary to maintain plasma and red cell folate concentrations similar to those found in breastfed infants

KEY WORDS Infants breastfeeding plasma folate red blood cell folate red blood cell status

Folate is necessary for normal growth (4 6) development (1) and function (2 28) of the human organism A prospective study on the folate status of infants and children related to other pertinent parameters was therefore undertaken If possible we wanted to define the optimal daily supply of the vitamin This is the first report from a series of studies made in a developed urban community We concentrate here on the folate levels in breastfed infants and demonstrate that the infants seem to be protected against folate deficiency during the nursing period Data illustrating the effects of normal physiological processes on plasma and red blood cell folate during the first year of life are also presented

MATERIAL AND METHODS

The material in this study consists of 35 full term infants borne by healthy mothers from the socio-economic middle class The mothers were given 0 2 g iron daily from the third month of gestation but no folic acid supplementation None of them developed anaemia during pregnancy or lactation

The infants were breastfed for at least 6 months and up to 14 months mean suckling period 7¾ months After weaning the infants were fed unboiled pasteurized cow s milk Vegetables iron fortified cereals containing 17 5 mg or more Fe per 100 g cereal meat and fish were gradually introduced at the age of 3 to 3½ months Vitamin supplementation with ascorbic acid (orange juice) and vitamin A and D (cod liver oil) was started one month after birth initially 5 mg ascorbic acid 3750 IU vitamin A and 375 IU vitamin D increasing to 15 mg 7500 IU and 750 IU respectively from 1½ months of age

The infants were examined regularly by one of us (J E) 3–5 hours after their last meal They were first seen within 7 days after birth thereafter at monthly intervals (+/− one week) On each occasion a clinical examination was performed and a capillary blood sample drawn The folate concentration in plasma and red blood cells was measured and the infants haemoglobin RBC VPRC red cell indices WBC reticulocytes and blood smears were studied

The adult reference material consisted of 50 female (non pregnant non lactating) and 50 male students and hospital staff aged 19 to 50 (mean 27½) years Venous blood samples were drawn 3–5 hours after their last meal

The haemoglobin values were obtained by a manual oxyhaemoglobin method (7 21) red and white blood cell counts by a Celloscope 401* (Linson Instrument AB

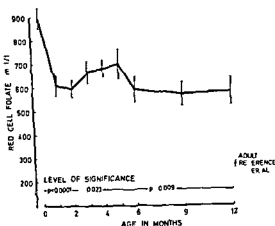


Fig 2 Red cell folate in 35 breastfed infants during first year of life Mean values ±1 S E M Statistical differences in red cell folate levels at various ages are denoted by levels of significance

($r=0\ 38$ $n=34$ $p<0\ 05$) a positive correlation was further observed between the plasma folate concentration at 4 months of age and that of the red blood cell folate concentration at 6 months ($r=0\ 53$ $n=35$ $p=0\ 001$) at 9 months ($r=0\ 49$ $n=33$ $p<0\ 01$) and at 12 months of age ($r=0\ 35$ $n=34$ $p<0\ 05$)

No significant correlation was found between the haemoglobin concentration and the concentration of folate in plasma and red cells at the ages of 0 3 6 9 and 12 months

DISCUSSION

It is well established that the plasma and red cell folate concentrations are higher in newborn full term infants than in their mothers and in adult reference materials (5 8 13) The present study confirms those observations Kamen & Caston (9) were able to demonstrate high concentrations of a folate binding protein in human umbilical cord serum different from that found in serum from pregnant women The mechanism by which the foetus is able to accumulate folate against a concentration gradient at the expense of the mother may thus include the existence of different folate binders in mother and foetus

During the whole nursing period the plasma and red cell folate values were higher than those recorded in the adult reference material In Israel however Matoth et al (16 17) found whole blood folate levels in breastfed infants to be of the same magnitude as in an adult reference material The reason for this discrepancy is not clear A folate binding protein is present in human milk in high concentrations (27) This protein may explain at least partly the high folate concentrations we have found in breastfed infants The folate values for the mothers were even lower during the lactation period (5) than during pregnancy

The infants were in negative folate balance during the first 2 months of life as judged from the decrease in red cell folate during that period The infants then went into a period of positive folate balance Landon & Oxley (13) observed a similar decrease in plasma folate during the first months of life irrespective of the initial folate concentrations but they did not state the type of feeding during that period Matoth et al (16) did not observe any differences in whole blood folate levels at different ages in breastfed infants They did not however observe the infants from birth nor did they follow the same group of infants at different ages Several factors may contribute to the observed variations in folate levels such as low folate concentrations in colostrum as compared with mature milk (11) rapid tissue uptake (22) and reduced tubular reabsorption (12) in the newborn infant

During the last part of the nursing period the folate values in plasma and red cells were lower than the preceding values probably due to either decreased intake or to increased demands The change in infant feeding during this period is probably significant a reduction in the relative importance of breast milk as foods containing less folate are introduced

A positive correlation between the plasma and red blood cell folate has previously been shown in adults (14) Our study demonstrates that this relationship can also be found in infancy The observed correlation between

Table 1 *Red blood cell status in breastfed infants during first year of life expressed as mean values with S D*

A R M =adult reference material S D =standard deviation

Age (months)	No of obs	Hb (g/dl)	RBC (×10¹²/l)	VPRC (l/l)	MCV (fl)
0	34	17 7±1 62	5 53 ±0 50	0 578±0 060	104 7 ±8 9
1	35	14 2±1 37	4 53 ±0 48	0 417±0 041	92 7 ±8 2
2	35	12 1±0 91	3 96 ±0 38	0 356±0 027	90 4 ±7 6
3	35	12 0±0 80	4 15 ±0 34	0 357±0 022	86 6 ±6 0
4	35	12 2±0 71	4 32 ±0 48	0 363±0 023	84 2 ±6 8
5	35	12 3±0 75	4 47 ±0 45	0 370±0 020	83 5 ±6 6
6	35	12 1±0 74	4 36 ±0 47	0 367±0 022	84 9 ±9 1
9	34	12 4±0 77	4 60 ±0 43	0 373±0 021	82 0 ±7 6
12	34	12 7±0 80	4 68 ±0 44	0 380±0 022	81 9 ±6 7
A R M female	50	13 6±0 64	4 48 ±0 33	0 411±0 022	92 0 ±5 8
A R M male	50	15 4±0 62	4 95 ±0 33	0 457±0 021	92 6 ±5 3

34 observations

Sweden) and the VPRC by using a microhaematocrit centrifuge for 3 minutes at 9400 rev/min at a mean of 6300 g Heparinized capillary tubes were used The haematological analyses were controlled by means of DADE* CH 60™ *Abnormal and Normal Haematology* Reference Serum and red blood cell folate were determined as *Lactobacillus casei* activity (15)

All results were analysed by BMDP statistical computer programs (3) The difference between groups of observations was tested by the Mann Whitney test

RESULTS

The growth of the infants was normal and did not differ from the standards established for Norwegian (25) and Swedish infants (10)

The haematological data are presented in Table 1 The values found in the 50 adult males used as controls were compatible with those reported for Norwegian men by Natvig & Vellar (20) The reference values found in adult females were compatible with those reported in Norwegian women without iron supplementation (20)

The plasma folate concentrations (Fig 1) were higher than in the adult reference material throughout the period of observation ($p<0\ 001$) The red cell folate concentrations (Fig 2) were also higher than in the adult reference material during the first year of life ($p<0\ 001$)

The correlation between plasma and red blood cell folate was studied A positive correlation was found between plasma and red cell folate at the age of one month ($r=0\ 40$ $n=35$ $p<0\ 05$) at 3 months ($r=0\ 34$ $n=35$ $p<0\ 05$) at 5 months ($r=0\ 63$ $n=35$ $p<0\ 001$) at 6 months ($r=0\ 50$ $n=35$ $p<0\ 01$) and at 9 months ($r=0\ 45$ $n=33$ $p<0\ 01$) A positive correlation was also observed between the plasma folate concentration at 5 months of age and that of the red blood cell folate concentration at 6 months ($r=0\ 36$ $n=35$ $p<0\ 05$) as well as between the plasma folate at 9 months and that of red cell folate at 12 months of age

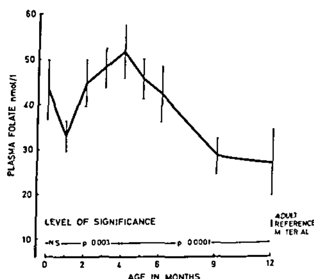


Fig 1 Plasma folate in 35 breastfed infants during first year of life Mean values ±1 S E M (Standard Error of the Mean) *Statistical differences in plasma folate levels* at various ages are denoted by levels of significance N S =not significant

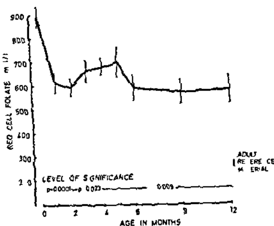


Fig 2 Red cell folate in 35 breastfed infants during first year of life Mean values ±1 S E M Statistical differences in red cell folate levels at various ages are denoted by levels of significance

($r=0\ 38$ $n=34$ $p<0\ 05$) a positive correlation was further observed between the plasma folate concentration at 4 months of age and that of the red blood cell folate concentration at 6 months ($r=0\ 53$ $n=35$ $p=0\ 001$) at 9 months ($r=0\ 49$ $n=33$ $p<0\ 01$) and at 12 months of age ($r=0\ 35$ $n=34$ $p<0\ 05$)

No significant correlation was found between the haemoglobin concentration and the concentration of folate in plasma and red cells at the ages of 0 3 6 9 and 12 months

DISCUSSION

It is well established that the plasma and red cell folate concentrations are higher in newborn full term infants than in their mothers and in adult reference materials (5 8 13) The present study confirms those observations Kamen & Caston (9) were able to demonstrate high concentrations of a folate binding protein in human umbilical cord serum different from that found in serum from pregnant women The mechanism by which the foetus is able to accumulate folate against a concentration gradient at the expense of the mother may thus include the existence of different folate binders in mother and foetus

During the whole nursing period the plasma and red cell folate values were higher than those recorded in the adult reference material In Israel however Matoth et al (16 17) found whole blood folate levels in breastfed infants to be of the same magnitude as in an adult reference material The reason for this discrepancy is not clear A folate binding protein is present in human milk in high concentrations (27) This protein may explain at least partly the high folate concentrations we have found in breastfed infants The folate values for the mothers were even lower during the lactation period (5) than during pregnancy

The infants were in negative folate balance during the first 2 months of life as judged from the decrease in red cell folate during that period The infants then went into a period of positive folate balance Landon & Oxley (13) observed a similar decrease in plasma folate during the first months of life irrespective of the initial folate concentrations but they did not state the type of feeding during that period Matoth et al (16) did not observe any differences in whole blood folate levels at different ages in breastfed infants They did not however observe the infants from birth nor did they follow the same group of infants at different ages Several factors may contribute to the observed variations in folate levels such as low folate concentrations in colostrum as compared with mature milk (11) rapid tissue uptake (22) and reduced tubular reabsorption (12) in the newborn infant

During the last part of the nursing period the folate values in plasma and red cells were lower than the preceding values probably due to either decreased intake or to increased demands The change in infant feeding during this period is probably significant a reduction in the relative importance of breast milk as foods containing less folate are introduced

A positive correlation between the plasma and red blood cell folate has previously been shown in adults (14) Our study demonstrates that this relationship can also be found in infancy The observed correlation between

plasma folate at one age and red cell folate later on implies that the red cell folate probably refelcts the amount of folate available in the bone marrow for deposition in the red cell during the development of the cell

Compared with the results reported by Moe (19) we found the haemoglobin concentration about 0.6 g/100 ml and the red blood cell count about 0.2×10^{12} /l higher and the VPRC about 0.02 l/l lower than Moe Methodological differences may well partially explain the differences Moe however found a mean MCV 7–12% higher than we did This could indicate a slight tendency to megaloblastosis in his material At the time of his study the milk formulas were not supplemented with folic acid

During the last part of pregnancy and lactation there are mechanisms in function which within certain limits provide the foetus and suckling infant with folate at the expense of the mother In this country nutritional folate deficiency in otherwise healthy women is very rare and folic acid supplementation is therefore not recommended We believe that the folate status of women in Norway is adequate and that the foetuses and infants are usually assured an adequate supply

The daily requirement of folate has been calculated from the folate content in human milk (16) This is an approach which may have certain limitations regarding artificial nutrition in infancy as we know that several nutrients are better utilized from human milk than from cow s milk preparations e g iron (18) and ascorbic acid (23) In a milk formula the amount of folate finally available for metabolic purposes will depend on factors such as the content of the vitamin in the raw material the nature of the potentially supplemented vitamin the effect of the manufacturing procedure storage and preparation before use as well as on the biological availability and absorption

A nutritional folate deficiency may well be present without any gross biochemical physiological or morphological disturbances The amount of the vitamin necessary to maintain a normoblastic erythropoiesis (24 26) may therefore not be optimal

It is reasonable to believe that an optimal tissue saturation of folate is present in normal breastfed infants providing the mothers folate status is adequate We suggest that the daily requirement during early infancy is the amount of folate necessary to maintain plasma and red cell folate concentrations similar to those found in breastfed infants

ACKNOWLEDGEMENTS

We are grateful for the invaluable assistance by Head Nurse Lovise Hoston and her staff at the Well Baby Clinic Department of Pediatrics We are also indebted to Kjeld Eldhuset B Sc at the Computer Department for computer assistance to Helge Erik Solberg M D at the Department of Clinical Chemistry for statistical advice and to Unni Fereváag for technical assistance

REFERENCES

1 Arakawa T Mizuno T Honda Y Tamura T Watanabe A Komatsushiro M Takagi T Iinuma K & Yamauchi N Longitudinal study on maturation patterns of EEG basic waves of infants fed on milk from mothers with low serum folate level *Tohoku J Exp Med 102* 81 1970

2 Davidson G P & Townley R R W Structural and functional abnormalities of the small intestine due to nutritional folic acid deficiency in infancy *Pediatrics 90* 590 1977

3 Dixon W J *BMDP Biomedical Computer Programs* University of California Press Berkeley 1975

4 Ek J The clinical importance of folate deficiency in coeliac disease in infancy and childhood In *Proc 15th Internat Congr Pediatr* New Delhi Oct 23–29 1977 Indian Academy of Pediatrics Bombay Abstr no 0602/03

5 Ek J & Magnus E M Folic acid levels in infancy In *Proc 14th Internat Congr Pediatr* Buenos Aires Oct 3–9 1974 Panamericana Buenos Aires 1974 *3* 49

6 Gandy G & Jacobson W Influence of folic acid on birthweight and growth of the erythroblastotic infant III Effect of folic acid supplementation *Arch Dis Child 52* 16 1977

7 Harboe M Studies on the conversion of oxyhemoglobin to alkaline hematin in dilute alkaline solutions *Scand J Clin Lab Invest 11* 138 1959

8 Hibbard E D Plasma and erythrocyte folate concentrations in normal mature infants *Arch Dis Child 48* 743 1973

9 Kamen B A & Caston J D Purification of folate binding factor in normal umbilical cord serum *Proc Natl Acad Sci USA 72* 4261 1975

10 Karlberg P Engstrom I Lichtenstein H &

Svennberg I The development of children in a Swedish urban community A prospective longitudinal study III Physical growth during the first three years of life *Acta Paediatr Scand* Suppl *187* 48 1968

11 Karlin R Sur les variations du taux d acide folique total dans le lait de femme au cours de la lactation Comparaison avec le lait bovin *C R Soc Biol (Paris)* *160* 21 3 1966

12 Landon M J & Hey E N Renal loss of folate in the newborn infant *Arch Dis Child* 49 79 1974

13 Landon M J & Oxley A Relation between maternal and infant blood folate activities *Arch Dis Child 46* 810 1971

14 Magnus E Folate studies *Scand J Haematol* Suppl 24 1975

15 Magnus E M In preparation

16 Matoth Y Pinkas A & Sroka Ch Studies on folic acid in infancy III Folates in breast fed infants and their mothers *Am J Clin Nutr 16* 356 1965

17 Matoth Y Pinkas A Zamir R Mooallem F & Grossowicz N Studies on folic acid in infancy I Blood levels of folic and folinic acid in healthy infants *Pediatrics 33* 507 1964

18 McMillan J A Landaw S A & Oski F A Iron sufficiency in breast fed infants and the availability of iron from human milk *Pediatrics 58* 686 1976

19 Moe P J Normal red blood picture during the first three years of life *Acta Paediatr Scand 54* 69 1965

20 Natvig H & Vellar O D Studies on hemoglobin values in Norway VIII Hemoglobin hematocrit and MCHC values in adult men and women *Acta Med Scand 182* 193 1967

21 Sheard C & Sanford A H Photo-electrometer with one stage of amplification *JAMA 93* 1951 1929

22 Shojania A M & Hornady G Folate metabolism in newborns and during early infancy II Clearance of folic acid in plasma and excretion of folic acid in urine by newborns *Pediatr Res 4* 422 1970

23 Snelling C E The plasma ascorbic acid of infants and children *J Pediatr 15* 824 1939

24 Sullivan L W Luhby A L & Streiff R R Studies of the daily requirement for folic acid in infants and the etiology of folate deficiency in goat s milk megaloblastic anemia *Am J Clin Nutr 18* 311 1966

25 Sundal A The norms for height (length) and weight in healthy Norwegian children from birth to 15 years of age *Univ Bergen Årbok* Med Series no 1 3 1956–1957

26 Velez H Ghitis J Pradilla A & Vitale J J Cali Harvard nutrition project I Megaloblastic anemia in kwashiorkor *Am J Clin Nutr 12* 54 1963

27 Waxman S Folate binding proteins *Br J Haematol 29* 3 1975

28 Zuelzer W W & Rutsky J Megaloblastic anemia of infancy *Adv Pediatr 6* 243 1953

Submitted April 11 1978
Accepted Aug 30 1978

(J E) Department of Paediatrics
Rikshospitalet
Oslo 1
Norway

Acta Pædiatr Scand 68 245–250 1979

ROLE OF PROLONGED BREAST FEEDING IN INFANT GROWTH

U M SAARINEN and M A SIIMES

From the Children s Hospital University of Helsinki Helsinki Finland

ABSTRACT Saarinen U M and Siimes M A (Children s Hospital University of Helsinki Finland) Role of prolonged breast feeding in infant growth Acta Paediatr Scand 68 245 1979.—The growth of 238 healthy full term infants was followed under a carefully monitored nutritional protocol during the first year of life The infants were weaned at different ages either to a proprietary infant milk formula or to a home-prepared cow s milk formula Solid foods were introduced at 3 5 months of age The 56 infants who were breast fed for a period of at least 6 months were compared to infants weaned prior to one month of age to one of the two milk regimens In the breast fed infants the weight weight for height age and skinfold thickness were similar to values in the proprietary formula fed infants but were lower than the corresponding values in the cow s milk fed infants at 6 months of age and subsequently By using weight for height age as a criterion no obesity was found among any of the 238 infants and only 1 7 % were considered to be overweight The results indicate that present recommendations for infant feeding in Finland—including prolonged breast feeding the use of proprietary milk formulas after weaning and later introduction of solid foods—prevent over nutrition

KEY WORDS Breast feeding infant nutrition milk obesity skinfold thickness

Obesity is the most common nutritional disorder in developed countries Since the treatment of established obesity is difficult more attention has been directed to its prevention

A high prevalence of excessive weight has been reported in infancy (10 12) with the use of cow s milk based formulas and early introduction of solid foods (7) Presumably such diets predispose to a parent induced calorie excess that is easier to avoid *in breast fed infants* In Finland the dietary instructions given by well baby clinics are followed *in* detail by the majority of parents This should make it possible to influence calorie intake and avoid overnutrition by appropriate recommendations for infant feeding

The benefits of breast feeding are currently being re emphasized for a variety of nutritional psychological and economic reasons (6) The new national recommendations for infant feeding in Finland given in 1975 stress the use of prolonged breast feeding delayed introduction of solid foods use of water instead of milk or sugar containing fluids for alleviating thirst and avoidance of additional salt

In the present study we followed the growth and certain aspects of the nutritional state of *breast fed infants* in a controlled manner The infants adherence to the above mentioned recommendations were monitored in a separate nutrition oriented well baby clinic After weaning either a proprietary infant milk for*mula* or a home prepared cow s milk formula was used The growth of breast fed infants was compared with that of bottle fed ones and with the available growth charts (2)

SUBJECTS

The group of 256 healthy newborn infants had gestational ages between 38 and 42 weeks and a birthweight over 3 0 kg They were born at the Helsinki University Central Hospital Departments of Obstetrics I and II during the first three months of 1975 All infants whose parents were

16 78

two bottle fed control groups were selected as follows (Fig 1)

1 B ust milk grou p Breast milk was the only source of milk in 56 infants until at least the age of 6 months after which they were gradually weaned to proprietary formula or low fat cow s milk (Fig 1)

Formula gr n p Proprietary formula feeding was tarted in 47 infants before the age of one month and con tinued until one year of age Partial breast feeding was continued to an average of 3 months of age and then dis continued (Fig 1)

3 C s milk g p Cow s milk formula prepared at home was started prior to the age of one month in 9 in fants and continued until the age of 6 months after which ommercially available low fat cow s milk was used Par tial breast feeding was continued in this group until an average of ? 5 months of age (Fig 1)

The average daily amounts of milk ingested by the in fants were estimated by the mothers and recorded on the history forms under the options of under 00 00–400 400–600 600–800 800–1000 and over 1000 ml/day No signifi ant differences were found between the two bottle fed groups by this method

METHODS

The weight supine length and skinfold thickness were measured in all infants by the same physician The weight was taken with the infants naked on a single calibrated ale Supine length was measured with the infant in a tret hed position on a special table fitted with a sliding footboard after the baby had been straightened the legs tretched the toes turned upwards and the sliding board then brought into contact with the heels The thicknesses of the triceps and subscapular skinfolds were measured at the ages of 6 9 and 1? months with a Harpenden skin fold caliper using the technique of Tanner & Whitehouse (15) A mean value of three consecutive measurements was used

C np ms i th gro th charts

The individual values for weight and length at different ages were plotted on magnified growth charts (?) and ex pressed as standard deviation scores In this manner we were able to eliminate the error that would arise if an in fant was not seen on the exact date of the age category This deviation in age was less than 4 days at 2 weeks of ge les than ? days up to and including 4 months of age nd les than weeks at the later ages

After the statistical analyses the final results were onverted back to cm and kg

D fn t m f bes t verweight th nn s d underw ight

The clas ificatic n is based on the method recommended by Smith & Wilkinson (11) The weight for height age was obtained by determining the age at which the infant s actual height falls on the 50th percentile (?) and then find ing the mean weight corresponding to that age The actual weight was compared to this mean we ght and expressed as a standard deviation score Obesity was defined as weight for height age above +. S D and overweight be tween +1 S D and +? S D Similarly thinness was de fined as a weight for height age below −? S D and underweight between −1 S D and −2 S D

Statistical analyses

In order to select the appropriate statistical methods we determined whether measurements followed a Gaussian distribution by using a chi square test All distributions were normal for weight and supine length though skewed distributions for weight have been reported elsewhere (14 16) Thus it was appropriate to use Student s t test in these statistical analyses For skinfold thickness values the chi square test gave skewed distributions only in cer tain instances in the formula group e g the subscapular skinfold at 9 months and the triceps skinfold at 1? months of age Because significant differences were found only between the breast milk group and the cow s milk group for data with normal distributions the Student s t test was used for these comparisons Analysis of variance which does not require a Gaussian distribution was used in addition

RESULTS

The values for body length in the breast milk group and the two bottle fed groups are shown in Table 2 The breast milk group dif fered significantly from the cow s milk group only if boys were separately analysed at 9 months of age ($p<0\ 02$) and at 12 months of age ($p<0\ 05$) The infants in all three groups were taller than the reference values (2) during the latter half of infancy

The body weight of the three milk groups is shown in Table 2 Infants of the breast milk group weighed significantly less than infants of the cow s milk group Among the girls differences were found at ages 4 ($p<0\ 05$) 6 ($p<0\ 005$) 9 ($p<0\ 005$) and 12 months ($p<0\ 01$) and in the boys at ages 6 ($p<0\ 05$) 9 ($p<0\ 005$) and 12 months ($p<0\ 01$) The girls in the formula group weighed less than the girls in the cow s milk group at ages 4 6 9 and 12 months ($p<0\ 01$ or $p<0\ 02$) but there were no corresponding significant differences among the boys The weight gains in the breast milk group and the formula group were similar in both sexes

The weight differences between the three milk groups during the first year of life are ex pressed in grams in Fig 2 where the mean

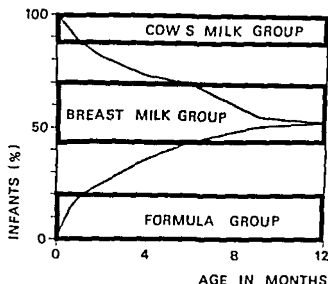


Fig 1 Milk feeding of 238 infants The darker shaded area shows the proportion of infants entirely on breast milk Partial breast feeding continued after the initiation of one of the two other milks as indicated by the lighter zones on both sides of the breast milk area The borders indicate how the breast milk group cow s milk group and formula group were formed

willing to join the program were included and were examined at 2 weeks 1 2 4 6 9 and 12 months of age The final series consisted of 238 infants 126 boys and 112 girls since 18 of the 256 infants dropped out of the study during the course of the year

Solid foods and supplementation

Solid foods were introduced according to a strict protocol and monitored by history and careful instructions at each visit as follows cooked vegetables and fruit at 3 5 months cereals at 5 months meat and eggs at 6 months and a normal mixed diet at 9 months of age This represents a later introduction of solid foods than has been customary in Finland and most other developed countries in recent years We also stressed the use of water instead of milk for thirst No sugar was added to the water except during acute febrile illnesses and no additional salt was used before 8 months of age No supplemental iron was given to the infants fed on breast milk or cow s milk but the proprietary formula was supplemented with 11 mg of elemental iron per liter At 2 weeks of age daily vitamin supplementation was started vitamin D 1000 IU vitamin A 1500 IU and vitamin C 20 mg

Milk feeding

All mothers were advised to use breast milk initially as the only source of milk This recommendation resulted in breast milk being used exclusively by 138 infants until months 92 infants to 4 months 56 infants to 6 months and 8 infants to 9 months of age (Fig 1) This incidence of breast feeding was much higher than what was recently estimated to be customary among the general infant population in Finland e g a 9% incidence of breast feeding at 6 months of age in the Helsinki area in 1966 (8)

Infants taking part in the study were identified by sequential numbers at the time of birth The infants given an odd number were later weaned to proprietary milk formula (Table 1) and those with an even number were weaned to a home prepared cow s milk formula For infants under 6 months of age the latter formula was prepared as follows 600 ml of commercially available pasteurized homogenized dairy milk (3 9% fat) and 400 ml of water were mixed and brought to boiling point after which 50 g lactose was added (Table 1) For infants over 6 months of age commercially available low fat (2 5%) milk was used as such (Table 1) Partial breast feeding was continued as indicated in Fig 1 The daily amount of milk recommended was one fifth of the infant s weight up to 1 liter

The analysis of the data revealed that those infants belonging to the groups with odd numbers were generally weaned somewhat earlier than the infants with even numbers (Fig 1) This difference was thought to be purely coincidental However one might speculate that the difference could be partly influenced by a certain degree of unwillingness on the part of the mothers to start their baby on home prepared cow s milk formula which perhaps was regarded as a somewhat old fashioned and inconvenient regimen This attitude might have predisposed to prolong breast feeding

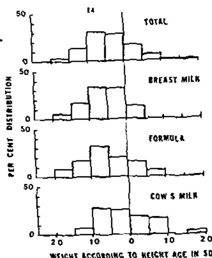
Study groups

From the total of 238 infants one breast fed group and

Table 1 *Composition of milks used in the study*

In the proprietary formula also vitamins and minerals were added which are not shown in the table

	Breast milk	Proprietary formula	Home prepared cow s milk formula	Commercial low fat cow s milk
Energy (kcal/l)	680	675	670	550
Lactose (g/l)	70	72	80	45
Proteins (g/l)	11	15	20	34
Casein (%)	40	40	80	80
Whey (%)	60	60	20	20
Fats (g/l)	36	35	23	25
Saturated fatty acids (%)	~50	36	73	73
Unsaturated fatty acids (%)	~50	64	27	27



F ʒ 3 Distribution and mean of weight for height age c ores in the entire series and in the three milk groups The weight for height age value was determined for each infant individually at one year of age and expressed as a standard deviation score Entire series (total) $n=?38$ Breast milk group $n=56$ Formula group $n=47$ Cow s milk group $n=9$

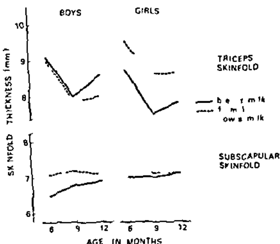


Fig 4 Thickness of the triceps and subscapular skinfolds at the ages of 6 9 and 1? months The lines indicate the mean values of the study groups shown for boys and girls separately

age (Student s t test $p<0\ 001$ analysis of vari ance F test $p<0\ 01$) The thicknesses of the triceps and subscapular skinfolds are shown separately for boys and girls (Fig 4) and in dicate the same differences in each sex The girls had slightly thicker subscapular skinfolds than the boys although this difference was of statistical significance only at 6 months of age (analysis of variance F test $p<0\ 05$)

DISCUSSION

In this investigation we compared the growth of infants who were breast fed for a period of at least 6 months with infants fed on two other milk regimens All infants followed the same regimen for solid foods

In early infancy there were only small dif ferences among the groups of infants on the three regimens The breast fed infants gained slightly more weight than those fed cow s milk based formulas but not to a statistically sig nificant degree During the latter half of in fancy the breast milk group was found to be lowest in body weight

The weight gain of infants fed proprietary formula was similar to that of the breast fed in fants This finding contradicts the commonly held notion that bottle feeding always results in a greater weight gain than breast feeding (5) and is in accord with earlier observations that artificially fed infants can gain weight at ap proximately the same rate as breast fed infants (9)

None of the infants were malnourished as noted during frequent physical examinations and as shown by a normal growth in length In fact the infants were somewhat taller on the average than the reference norms for Finnish babies obtained 15 to 20 years ago (2)

The infants of the cow s milk group had the highest weight weight for height age and skinfold thickness Because no differences were noted in the volumes of milk consumed and since the energy content of the commer cial low fat cow s milk used after 6 mon hs of age was lower than that of breast milk or the proprietary formula the diff rences in growth might be due to the composition of these milks and especially in respect to the protein and fat composition

There is a widespread tendency to introduce non-milk foods at an early age although no

Table 2 *Supine length in cm (mean ± S D left) and weight in kg (mean ± S D right) of the breast fed infant group and the two bottle fed control groups*

Age (mo)	Breast milk Boys	(n=33)	Girls	(n=23)
0	50 7±1 6	3 7±0 4	50 1±1 6	3 5±0 3
0 5	53 7±2 1	3 8±0 5	52 8±1 7	3 6±0 3
1	55 9±2 2	4 4±0 6	55 0±1 5	4 1±0 3
2	59 2±2 2	5 4±0 6	58 3±1 9	5 0±0 3
4	65 3±2 1	6 9±0 9	63 7±1 7	6 5±0 4
6	68 8±2 4	7 8±0 9	67 7±2 0	7 4±0 6
9	73 4±2 3	9 0±1 0	72 7±1 8	8 5±0 6
12	77 5±2 4	10 0±0 9	76 5±2 0	9 5±0 8
	Cow s milk Boys	(n=13)	Girls	(n=16)
0	50 4±1 3	3 8±0 3	49 8±2 1	3 5±0 4
0 5	53 4±1 2	3 7±0 4	52 5±1 6	3 6±0 4
1	55 3±1 3	4 2±0 5	54 6±1 7	4 0±0 5
2	59 0±1 6	5 2±0 5	58 2±1 7	4 9±0 5
4	65 3±1 6	7 1±0 9	64 3±1 3	6 8±0 4
6	69 3±2 0	8 4±0 9	68 3±1 6	7 9±0 4
9	75 1±1 8	10 0±1 0	73 5±1 7	9 2±0 7
12	79 2±2 3	11 0±1 1	77 2±2 2	10 3±0 9
	Formula Boys	(n=29)	Girls	(n=18)
0	51 4±1 7	3 8±0 4	49 5±1 8	3 4±0 3
0 5	53 6±2 0	3 8±0 4	52 5±1 7	3 5±0 4
1	56 0±2 0	4 3±0 4	54 6±1 6	3 9±0 3
2	59 4±2 4	5 4±0 6	57 8±1 5	4 7±0 4
4	65 6±2 3	7 2±0 7	63 4±1 5	6 3±0 6
6	69 6±2 2	8 3±0 8	67 9±1 9	7 3±0 8
9	74 4±2 4	9 4±0 8	72 1±1 8	8 5±0 9
12	78 1±2 5	10 3±0 9	76 6±2 3	9 4±1 0

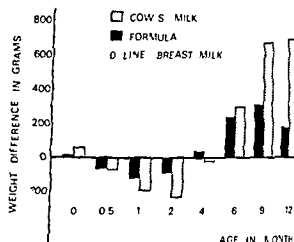


Fig 2 Development of weight differences between breast milk group and the two bottle fed control gro[ups] during the first year of life Values of the brea[st milk] group are used as reference standard and represent zero line

weight of the breast milk group represents the baseline In early infancy the breast fed infants gained slightly more weight than the bottle fed infants although these differences did not reach statistical significance After 4 months of age there was a gradual change in the opposite direction

The mean weight for height age and its distribution at one year of age is shown in Fig 3 for each of the three milk groups as compared to the entire series of 238 infants using the distribution of individual standard deviation scores The mean weight for height age of [the] entire series was −0 43 S D In the breast m[ilk] group the mean score was −0 69 S D and the formula group −0 62 S D The mean va[lue] of −0 09 S D of the cow s milk group w[as] higher than those of the two other gro[ups] ($p<0$ 001) and this difference also was sig[nifi]cant using the nonparametric U test of Ma[nn] & Whitney

At one year of age none of the infants in [the] breast milk or formula groups were ov[er]weight but 1 out of 29 infants in the cow s m[ilk] group was In the entire series no infant w[as] found to be obese and 4 out of 238 were ov[er]weight Of these 4 infants 2 had been wean[ed] to cow s milk and 2 to formula prior t[o] months of age

The proportion of underweight infants [ac]cording to the criteria used was 22% in [the] breast milk group 26% in the formula gro[up] and 6 9% in the cow s milk group at one y[ear] of age None was found to be thin

The infants of the cow s milk group w[ere] found to have the most subcutaneous fat [in] measurements of the triceps and subscapu[lar] skinfolds (Fig 4) However the difference b[e]tween the cow s milk group and the brea[st] milk group was only statistically significant [in] the triceps skinfold thickness at 12 months

Acta Pædiatr Scand 68 251–256 1979

GROWTH HORMONE RESPONSE TO PROSTAGLANDIN E

W HAMILTON and D M HUSSEIN

From the University Department of Child Health Royal Hospital for Sick Children Glasgow UK

ABSTRACT Hamilton W and Hussein D M (University Department of Child Health Royal Hospital for Sick Children Yorkhill Glasgow) Growth hormone response to prostaglandin E. Acta Paediatr Scand 68 251 1979 —C AMP causes release of growth hormone from the pituitary Prostaglandin increases pituitary adenylate cyclase and C AMP and therefore acts like a growth hormone-releasing hormone Using PGE_2 (30 μg per kilo body weight) given intravenously we have demonstrated that in no case was there a growth hormone response to insulin hypoglycaemia when PGE_2 failed to evoke a response However in 6 of 18 patients unresponsive to insulin hypoglycaemia a significant rise in growth hormone was obtained from PGE_2 We argue that in these 6 patients hypothalamic unresponsiveness to hypoglycaemia must be operative whereas PGE_2 acting directly at pituitary level is likely to have caused the release of preformed growth hormone from the pituitary Administration of PGE_2 does not cause hypoglycaemia but rather a slight rise in the plasma glucose level Thus the risk of brain damage which is inherent in the insulin hypoglycaemia test is avoided

KEY WORDS Growth hormone insulin hypoglycaemia test prostaglandin stimulation

A number of tests have been designed to evaluate the pituitary reserve of growth hormone These have been evaluated by Frasier (2) Good screening tests detect all patients who are likely to have inadequate growth hormone reserve while the ideal definitive test detects only patients who are growth hormone deficient However it would appear that such tests as insulin hypoglycaemia arginine infusion and oral L dopa all highly satisfactory according to the above criterion for a definitive test utilize hypothalamic mechanisms to increase circulation of growth hormone levels Hypoglycaemia acting through glucoprivation at the lateral hypothalamus results in release of growth hormone releasing hormone (GH RH) which however still awaits identification and characterization L Dopa is converted in the brain to dopamine and via dopaminergic receptors at the lateral border of the ventromedial nucleus stimulates the release of GH RH Arginine and glycine probably act as neuroinhibitors of cells in the median eminence decreasing the release of growth hormone release inhibitory hormone (GH RIH or somatostatin) These amino acid tests may therefore specifically detect growth hormone deficiency due to excessive inhibition of growth hormone release from the pituitary (1) Such a cause of short stature has not yet been demonstrated although the possibility has been suggested (1) If however a test agent were available to stimulate the pituitary to secrete growth hormone then separation of hypothalamic and pituitary causes for growth hormone deficiency would be possible Additionally at present it is not clear if a lack of response to the insulin hypoglycaemia test indicates that hypothalamo pituitary stimulation is defective or that the pituitary cells lack adequate growth hormone synthetic ability The therapeutic implications of such a test substance would be its possible use as a stimulating agent to induce pituitary output of preformed growth hormone if only hypothalamic mechanism were defective

Prostaglandins are known to increase growth hormone secretion (3 4) It is assumed

Present address El Maadi Hospital Cairo Egypt

evidence is available to indicate that this achieves a better nutritional state On the contrary it appears to result in excessive weight gain (13) In the 1950s when our reference norms (2) were obtained the use of home prepared cow s milk formulas was common in this country Our present dietary regimen differed from the customary practice in the 1950s and 1960s in several ways that might influence weight gain such as postponement of the introduction of solid foods use of sugar free water for thirst and avoidance of added salt Our data however do not offer sufficient evidence in support of the role of the above mentioned factors in infant growth since no essential differences were found between our cow s milk group and the reference norms in the weight gain weight for height age or proportion of overweight infants Studies on 1684 infants in Helsinki area in 1966 showed that 1 1% were obese and 5 4% were overweight at one year of age by using the same criteria for obesity and overweight as used in this study (8) compared to 3 4% of overweight infants in our cow s milk group

Successful breast feeding for 6 months or more can be achieved by mothers if they are actively encouraged and when the infants are closely followed by trained personnel Although a number of identifiable factors are involved in the pathogenesis of overnutrition we feel that prolonged breast feeding is a natural way to promote normal growth and possibly decrease the risk of later obesity (1 3 4)

ACKNOWLEDGEMENTS

Supported by grants from Foundation for Pediatric Research Finland and Foundation for Chemical Research Finland

REFERENCES

1 Asher P Fat babies and fat children The prognosis of obesity in the very young *Arch Dis Child* 41 672 1966

2 Backstrom L & Kantero R L Cross sectional studies of height and weight in Finnish children aged from birth to 20 years *Acta Paediatr Scand* Suppl 220 9 1971

3 Charney E Goodman H C McBride M Lyon B & Pratt R Childhood antecedents of adult obesity Do chubby infants become obese adults *N Engl J Med* 295 6 1976

4 Eid E E Follow up study of physical growth of children who had excessive weight gain in the first six months of life *Br Med J* II 74 1970

5 Fomon S J & May C D Metabolic studies of normal full term infants fed pasteurized human milk *Pediatrics* 22 101 1958

6 IPA Seminar Montreux Aug 15–20 1975 Recommendations to action programmes to encourage breast feeding *Acta Paediatr Scand* 65 275 1976

7 Jackson R L Westerfeld R Flynn M A Kimball E R & Lewis R B Growth of well born American infants fed human and cow s milk *Pediatrics* 33 642 1964

8 Kantero R L Unpublished observations 1966

9 Pava S I Pattern of growth of selected groups of breast fed infants in Iowa city *Pediatrics* II 8 1953

10 Shukla A Forsyth H A Anderson C M & Marwah S M Infantile overnutrition in the first year of life A field study in Dudley Worcestershire *Br Med J* IV 507 1972

11 Smith D W Growth and its disorders Basics and standards approach and classification growth deficiency disorders growth excess disorders obesity *In* A J Schaffer & M Markowitz (eds) *Major problems in clinical pediatrics* W B Saunders & Company Philadelphia 1977 vol XV p 2?

12 Sveger T Lindberg B Weibull B & Olsson U L Nutrition overnutrition and obesity in the first year of life in Malmo Sweden *Acta Paediatr Scand* 64 635 1975

13 Taitz L S Infantile overnutrition among artificially fed infants in the Sheffield region *Br Med J* I 315 1971

14 Tanner J M Physical growth and development *In* J O Forfar & G C Arneil (eds) *Textbook of Pediatrics* Churchill Livingstone Edinburgh and London 1973

15 Tanner J M & Whitehouse R H Revised standards for triceps and subscapular skinfolds in British children *Arch Dis Child* 50 142 1975

16 Tanner J M Whitehouse R H & Takaishi M Standards from birth to maturity for height weight height velocity and weight velocity British children 1965 *Arch Dis Child* 41 613 1966

Submitted April 13 1978
Accepted June 30 1978

(U M S) Children s Hospital
Stenbackinkatu 11
SF 00290 Helsinki 29
Finland

Table 2 *Insulin hypoglycaemia and prostaglandin E tests in ten children with short stature (both tests positive)*

Patient No /Sex	Age (years)	Plasma growth hormone (µg/l) IHT			PGE₂			Plasma glucose in response to PGE₂ (mmol/l)		
		0	Max	Time	0	0	60	0	20	60
13 F	7 4	4 5	30 0	60	2 25	21 0	25 8	0	2 4	3 3
14 M	8 7	7	30 0	90	0 9	1 1	11 4	1 7	1	3 3
15 M	9 7	0 9	20 6	60	1 5	7 5	15 5	1 6	2 6	2 6
16 F	10 0	13 5	30 0	5	10 5	8 5	11 1	2 0	2 4	2 1
17 M	10 0	4 0	21 2	0	16	21 8	14 2	1 15	1 4	2 0
18 M	11 8	14 7	23 5	90	13	13 5	13 0	1 6	1 5	2 3
19 M	13 3	3 3	23 9	30	4 4	11 4	30 0	2 1	2 4	2 3
0 M	14 7	1 5	30 0	90	14 3	11 0	30 0	1 7	1 9	2 1
1 M	16 7	1 5	21 3	60	1 1	3	15 8	3	8	2 8
2 M	16 9	30 0	30 0	60	11 4	30 0	30 0	1 5	2 3	2 1

therapy It will be further noted that in these 12 patients PGE did not provoke a significant increase in the circulating levels of growth hormone and neither did it cause hypoglycaemia but rather a slight rise in plasma glucose levels

In Table 2 are results from a further 10 patients in whom both tests gave an adequate growth hormone response Here again PGE did not provoke hypoglycaemia but in 5 out of 10 there was an increase (around 2 fold) in the blood glucose levels

In Table 3 are the results from 6 patients in whom hypoglycaemia failed to provoke a growth hormone response while the response to PGE_2 indicated adequate growth hormone reserve Two of these patients are now receiving growth hormone therapy Again in this group PGE_2 induced a slight increase in blood glucose levels

In Table 4 are the effects of PGE_2 on the plasma levels of TSH LH and FSH in those patients for whom sufficient plasma was available Only TSH levels showed an increase in 8 of 19 patients LH and FSH levels were unaffected in the 10 samples assayed

The overt clinical response to PGE varied from mild to moderate but immediate nausea vomiting abdominal discomfort headache and a desire to micturate and defaecate were

Table 3 *Insulin hypoglycaemia and prostaglandin E tests in six children with short stature (IHT negative PGE₂ positive)*

Patient No /Sex	Age (years)	Plasma growth hormone (µg/l) IHT			PGE			Plasma glucose levels in response to PGE[illegible] (mmol/l)		
		0	Max	Time	0	20	60	0	20	60
3 F	6 7	6 5	4 9	60	2 5	4 4	11 3	2 0	2 3	2 7
4 F	10 1	5 6	5 3	5	3	1 1	11 0	1 2	2 1	2 1
25 M	10 8	0 1	8 1	30	2 3	7 7	12 9	3 3	2 9	3 4
6 M	11 6	0 1	1 8	1 0	1 8	30 0	3 6	2 4	3 1	3 4
27 M	16 1	6 9	9	30	2 3	9 3	18 3	2 6	3 3	3 4
28 M	17 3	2 7	5 1	1 0	5 1	13 4	13 2	2 1	2 2	3 4

Patients now receiving growth hormone as therapy

Table 1. *Insulin hypoglycaemia and prostaglandin E_2 tests in twelve children with short stature (both tests negative)*

Patient No./Sex	Age (years)	Plasma growth hormone (µg/l) IHT 0	IHT Max	IHT Time	PGE₂ 0	PGE₂ 20	PGE₂ 60	Plasma glucose levels in response to PGE_2 (mmol/l) 0	20	60
1 F	7.5	4.8	3.4	120	3.4	2.8	5.3	3.4	4.5	4.8
2 M [a]	9.2	1.5	2.1	10	0.3	0.6	1.5	3.6	4.1	5.1
3 M	9.6	2.1	6.8	120	6.8	3.8	1.2	4.3	4.8	5.3
4 M	9.7	0.1	4.6	60	0.9	0.5	1.3	2.9	4.8	5.5
5 M	9.9	0.8	4.1	90	0.9	2.3	5.4	3.2	4.4	5.2
6 M	10.1	0.1	4.7	60	0.9	1.1	0.5	5.2	5.1	5.6
7 M [a]	10.5	0.8	0.3	120	2.5	2.4	0.8	3.1	5.0	4.8
8 F	10.9	0.3	0.9	120	0.9	1.1	0.5	5.2	5.6	6.1
9 M	11.3	3.6	5.3	60	3.5	2.0	0.3	3.3	4.0	4.6
10 M	11.9	0.1	6.3	60	5.9	5.7	7.0	4.0	4.6	5.2
11 M	14.1	0.2	2.8	60	0.3	1.5	3.0	3.1	5.1	6.8
12 M	15.0	0.3	0.4	60	0.3	1.4	0.1	4.2	5.4	5.8

[a] Patients now receiving growth hormone as therapy

by several workers that its action is through an increase in intracellular 3,5 cyclic AMP at pituitary level (5). This has led us to explore the value of prostaglandin (PGE_2) as a *test substance* for growth hormone reserve in children with short stature.

MATERIALS AND METHODS

The patients investigated were 21 boys and 7 girls whose ages ranged from 6.6 to 17.3 years. All were of short stature and by virtue of their anthropometric data and their lack of other organic growth retarding disease required endocrine investigation. The investigations were carried out by permission of fully informed parents.

The insulin hypoglycaemia test (IHT) was performed after an overnight fast. A slow intravenous infusion (0.45% saline) was established and at zero time soluble insulin (0.1 units per kilo body weight) was given intravenously. In the absence of clinical hypoglycaemic features at 30 min a second dose of insulin (0.1 units per kilo body weight) was given. Lithium heparinized blood samples were taken at 0, 5, 10, 20, 30, 60, 90 and 120 min. At 120 sample PGE_2 (30 µg per kilo body weight) was given intravenously (3) as an undiluted bolus. Further blood samples were taken at 140 and 180 using the 120 sample for the basal level for the PGE_2 response. PGE_2 (Upjohn Ltd, Crawley, England) was used. Growth hormone was assayed by a double antibody radioimmunoassay technique (CIS Kit, Eurotype Services Ltd, Finchley, N 12).

Plasma glucose levels were assayed by a Bechman analyser method. Retrospectively, when sufficient plasma remained, LH, FSH and TSH were assayed on the post PGE_2 samples. Double antibody radioimmunoassay methods were used (CIS Kit, Eurotope Services Ltd, Finchley, N 12) for these assays.

RESULTS

To interpret our data we have considered that absolute deficiency of growth hormone exists when the plasma growth hormone levels do not rise above 7 µg/l following adequate hypoglycaemia; partial deficiency is represented by plasma levels between 7 and 10 µg/l while values greater than 10 µg/l are regarded as representing normal growth hormone reserve. To assess the significance of the pituitary responsiveness to PGE_2 we have used these same plasma levels of growth hormone. Adequate hypoglycaemia is represented by plasma glucose levels not exceeding 1 mmol/l. Plasma glucose levels following insulin administration are not quoted since satisfactory hypoglycaemia was ensured in all patients.

In Table 1 are the levels of plasma growth hormone in response to insulin and PGE_2 in 12 patients unresponsive to the IHT. In each case only the zero and the peak values are given with the corresponding time interval for the peak value. The patients marked *a* have subsequently been started on growth hormone

response can be obtained in the face of a partial response from either alone The intravenous glycine test has been reported to give an adequate growth hormone response in 3 of 50 patients in whom the IHT was negative In 4 patients of the same series the glycine test was negative while the IHT was adequate (1) These authors suggest that glycine acts by inhibiting somatostatin activity at the pituitary level and not by stimulating GH RH The PGE_2 test would appear to supercede these amino acid tests since in our series there was no case responsive to the IHT and negative to PGE Here we argue that direct pituitary stimulation is a theoretical possibility accounting for our findings in those patients who did not respond to insulin hypoglycaemia but who did respond to PGE

Hypothalamic extracts stimulate adenylate cyclase activity and C AMP concentrations in the anterior pituitary (8) C AMP causes release of growth hormone from the pituitary (5) PGE_2 also increases pituitary adenylate cyclase and C AMP (5) Thus if PGE_2 fails to provoke an increased level of circulating growth hormone the inference is that there has been failure of growth hormone synthesis by the pituitary cells This distinction between failure of synthesis and failure of release of growth hormone is not made by utilizing a test which is mediated via the hypothalamus since it is not possible to determine whether or not failure to increase growth hormone from a hypothalamic mediated response indicates failure of hypothalamic responsiveness or lack of pituitary growth hormone reserve The PGE_2 test may therefore identify patients whose short stature is due to failure of adequate growth hormone synthesis

It will be noted in our regime that IHT and the PGE test have been performed at the same time Other workers have suggested that at least a 24 hour period should elapse between two pituitary stimulation tests thereby suggesting the possibility of pituitary exhaustion We do not think that this is relevant and our data would support that view Additionally the PGE test may be performed without an overnight fast and at any time of the day It is therefore particularly useful for patients in whom there is a history of convulsions or of spontaneous hypoglycaemic attacks In such patients failure to increase growth hormone levels to PGE_2 stimulation alerts the investigator to the need for consummate caution if an IHT is undertaken

TSH and ACTH are also released by prostaglandins but not LH nor prolactin (5) Our data show that PGE_2 stimulation does give a rise in plasma TSH levels (8 of 19) but no response was noted in LH or FSH levels (10 of 10) Our selection for the timing of plasma sampling in the PGE test was partly to conform to the recommended timing for the LRH/TRH test These times (0 20 60 min) seemed appropriate because the PGE test gives immediate predictable clinical responses which continue throughout the first twenty minutes post injection followed by a slow reduction in the intensity of the reactions until sleep ensures by sixty minutes

The PGE_2 test as described was unpleasant for all the children but in 6 the only side effect was transient pain at the injection site There are as far as we judge no risks and the test need never be repeated because of failure to achieve a clinical response PGE_2 does not cause hypoglycaemia so that the patient is at no risk from hypoglycaemic cerebral damage nor anoxia following convulsions

The PGE_2 test at present does not replace all other tests because growth hormone as therapy is still the only treatment when both the IHT and PGE_2 test are negative It is therefore still necessary to perform the IHT if the PGE_2 test is positive

It might be argued that the growth hormone response to PGE_2 was part of a systemic reaction and not specifically a pituitary response Notwithstanding this possibility intravenous prostaglandin is worthy of further investigation as a test procedure to separate hypothalamic from pituitary causes of growth hormone deficiency

Table 4 *Plasma levels of TSH LH and FSH in response to intravenous prostaglandin E*
UD = Undetected

Patient No /Sex	Age (years)	Plasma TSH (mU/l)			Plasma LH (U/l)			Plasma FSH (U/l)		
		0	20	60	0	20	60	0	0	60
23 F	6 7	6 0	5 5	4 4						
13 F	7 4	4 0	4	4	1 8	1 8	1 8	0 9	0 9	0 9
14 M	8 7	4 9	16 8	10 9						
2 M	9 2	2 2	6 7	2 1	3 1	3 1	3 1	0 7	0 7	0 7
3 M	9 6	2 1	2 1	2 1	3 1	3 1	3 1	0 7	0 7	0 7
4 M	9 7	UD	5 6	2 9	3 1	3 1	3 1	0 7	0 7	0 7
16 F	10 0	4 4	35 3	20 2						
6 M	10 1	2 6	2 1	2 1	3 1	3 1	3 1	1 0	1 0	1 0
7 M	10 5	5 3	8 2	5 8	3 1	3 1	3 1	0 7	0 7	0 7
25 M	10 8	6 9	5 7	6 0	3 1	3 1	3 1	0 7	0 7	0 7
8 F	10 9	2 1	11 7	2 1	3 1	3 1	3 1	0 7	0 7	0 [illegible]
26 M	11 6	2 9	3 4	2 9						
18 M	11 8	2 5	9 8	5 2	2 0	1 3	1 2	1 9	1 9	1 9
19 M	13 3	3 1	2 7							
20 M	14 7	1 6	1 5	1 5						
12 M	15 0	5 3	4 7	4 7	3 1	3 1	3 1	0 7	0 7	0 7
27 M	16 1	2 9	2 9	2 9						
21 M	16 2	1 5	1 5	1 5						
22 M	16 9	2 5	2 4	1 9						

noted All patients were significantly drowsy after the test and slept for up to 2 hours

DISCUSSION

It is generally accepted that the secretion of growth hormone occurs physiologically in response to sleep and to hypoglycaemia The insulin hypoglycaemia test (IHT) utilizes physiological hormone reflex arcs to induce growth hormone release from the pituitary It is however possible that altered hypothalamic responsiveness to gluco privation could result in lack of growth hormone releasing hormone (GH RH) or in a disproportionate amount of somatostatin thus resulting in a peripheral lack of growth hormone even although the pituitary reserve of growth hormone were adequate Were such a situation to obtain it should be possible to find a negative growth hormone response to hypoglycaemia but an adequate response following a test which directly stimulates the pituitary to release its growth hormone In theory therefore short stature attributable to lack of growth hormone could result from a lesion of the hypothalamus or from a lack of endogenous growth hormone in the pituitary At present there is no real clinical or biochemical separation of these possible aetiologies and treatment of both (were they to exist) would presently be by growth hormone administration

If however short stature were due to a hypothalamic abnormality where pituitary growth hormone reserve was of itself adequate then rational treatment would be the administration of a drug known to stimulate directly the pituitary to release its growth hormone

The data which we present here demonstrate that in those children tested there were some with the traditionally accepted growth hormone lack in response to hypoglycaemia but who showed a normal growth hormone response to PGE Conversely in this series the PGE test did not indicate lack of growth hormone reserve in any patients when the IHT gave an adequate response

Many authorities believe that a child should be demonstrated to have growth hormone deficiency on two different tests before administering growth hormone as therapy There is published evidence (7) that with the combined *l* arginine and L dopa test an adequate

Acta Pædiatr Scand 68 257–264 1979

SERUM CONCENTRATIONS OF THYROTROPIN THYROID HORMONES AND THYROID HORMONE BINDING PROTEINS DURING ACUTE AND RECOVERY STAGES OF IDIOPATHIC RESPIRATORY DISTRESS SYNDROME

B BROCK JACOBSEN B PEITERSEN and L HUMMER

From the University Clinic f Paediatrics Children s Hospital Fuglebakken a d from the Department of Nuclear Medicine Rigshospitalet Copenhagen Denmark

ABSTRACT Jacobsen B B Peitersen B and Hummer L (University Clinic of Paediatrics Children s Hospital Fuglebakken and the Department of Nuclear Medicine Rigshospitalet Copenhagen Denmark) Serum concentrations of thyrotropin thyroid hormones and thyroid hormone-binding proteins during acute and recovery stages of idiopathic respiratory distress syndrome Acta Paediatr Scand 68 257 1979.—A total number of 27 premature infants with idiopathic respiratory distress syndrome (IRDS) and 52 healthy controls with comparable gestational age and body weights were studied during the first month of life In infants with IRDS a reduced thyrotropin (TSH) response to birth was suggested as serum TSH was lower in IRDS patients than in controls during the first two days of life Low serum concentrations of thyroid hormones were found in the acute stage of IRDS reaching minimal values by day 3–5 After that period an increase in thyroid hormone levels occurred The serum T_3 increased to the level of healthy prematures by day 6–10 whereas the serum T_4 increased to normal levels by day 21–30 Serum concentrations of thyroxine-binding globulin (TBG) were significantly lower in IRDS patients than in healthy controls a gradual increase to normal levels occurred during recovery Serum prealbumin (TBPA) levels in IRDS infants increased rapidly after birth and exceeded levels of healthy infants Serum albumin values were not significantly different in the two groups of infants The serum T_4/TBG ratios were low during recovery from IRDS

KEY WORDS Thyrotropin thyroid hormones thyroxine binding globulin prealbumin albumin idiopathic respiratory distress syndrome

In 1974 studies by Redding & Pereira (24) showed a significantly decreased total serum thyroxine concentration in infants with idiopathic respiratory distress syndrome (IRDS) In 1976 Cuestas et al (5) reported that cord serum triiodothyronine also was lower in infants with IRDS than in those without These findings have been confirmed by others (1 17) The pathogenetic significance of the low thyroid hormone levels is obscure but studies in fetal and adult animals have indicated an important influence of the thyroid hormones upon lung growth and production of the pulmonal surfactant factor (8 23 29) Furthermore a relatively high incidence of IRDS in neonatal hypothyroidism in man has been reported (28)

The mechanisms that determine diminished thyroid hormone levels in IRDS infants are unknown but a decreased activity of the thyroid gland has been suggested (5 6 17 24) Recently we reported preliminary results indicating low serum thyroxine binding globulin concentration in IRDS infants (13 15) The changes in serum concentrations of thyroid hormones following recovery from IRDS have not been fully elucidated (1 6 18) and no studies of the thyroid hormone binding protein levels have been reported In the present study therefore we determined serum concentrations of thyroid hormones and hormone binding proteins in infants during the acute and recovery stages of IRDS and compared the results with those obtained in healthy prematures

MATERIALS AND METHODS

A total number of 27 premature infants with IRDS was studied The diagnosis of IRDS was based upon clinical biochemical and radiological data (16 26) The treatment was carried out in accordance with previous report (16)

ACKNOWLEDGEMENTS

We are indebted to Professor J H Hutchison for his encouragement in this work We acknowledge the valuable assistance of our technicians Mrs A Reid and Miss L Thomson Dr J G Ratcliffe RIA Unit Royal Infirmary Glasgow confirmed the growth hormone levels in duplicate samples of plasma from several patients Part of the equipment used in this work was obtained with money from the Rankin Memorial Funds of Glasgow University

REFERENCES

1 Florea I Popa M Simionescu L Dinulescu E & Juvine E Glycine intravenous load for diagnosis of growth hormone deficiency *Clin Endocrinol* 5 283 1976
2 Frasier D S A review of growth hormone stimulation tests in children *Pediatrics* 53 929 1974
3 Hertelendy F Todd H Ehrhart K & Blute R Studies on growth hormone secretion IV In vivo effects of prostaglandin E1 *Prostaglandins* 2 79 1972
4 Ito H Momose G Katayama T Takagishi H Ito I Nakajima H & Takei Y Effect of prostaglandin on the secretion of human growth hormone *J Clin Endocrinol Metab* 32 857 1971
5 MacLeod R & Lehmeyer J E Release of pituitary growth hormone by prostaglandins and dibutyryl adenosine cyclic 3 5 monophosphate in the absence of protein synthesis *Proc Natl Acad Sci* 67 1172 1970
6 Schofield J G Role of cyclic 3 5 AMP in release of growth hormone in vitro *Nature* 215 1382 1967
7 Weldon V V Clarke W L & Pachara A S Growth hormone release using arginine and L-dopa in combination *J Pediatr* 87 540 1975
8 Zor U Kaneko T Schneider H P G McCann S M Lowe I P Bloom G Borland B & Field J B Stimulation of anterior pituitary adenyl cyclase activity and adenosine 3 5 cyclic phosphate by hypothalamic extract and prostaglandin E1 *Proc Natl Acad Sci* 63 918 1969

Submitted May 22 1978
Accepted Sept 11 1978

(W H) University Department of Child Health
Royal Hospital for Sick Children
Yorkhill
Glasgow G3 8SJ
Scotland

Table 2 Median and range of serum T_4, T_3 and TSH concentrations in premature infants with idiopathic respiratory distress syndrome (IRDS) and in healthy controls

No = number of samples NS = not significant

	Postnatal age in days				
	<3	3–5	6–10	11–0	1–30
Serum T_4 (nmol/l)					
IRDS					
Median	83	64	96	107	109
Range	(46–167)	(6–1 0)	(45–13?)	(60–154)	(8?–167)
No	18	9	10	20	14
Controls					
Median	147	175	155	130	131
Range	(54– 89)	(54– 3)	(85– 70)	(80–?37)	(77–234)
No	35	17	9	49	25
	$p<0\ 001$	$p<0\ 001$	$p<0\ 001$	$p<0\ 0$	NS
Serum T_3 (nmol/l)					
IRDS					
Median	0 53	0 9	1 34	1 47	1 75
Range	(0 70– 4)	(0 0–0 39)	(0 61–1 86)	(0 96– 41)	(1 0 – 60)
No	14	4	7	13	10
Controls					
Median	1 0	1 31	1 5	1 78	1 83
Range	(0 11–? 33)	(0 8 – 11)	(0 37–4 03)	(0 86–4 ?4)	(0 66–3 7?)
No	6	10	1	?7	?
	$p<0\ 05$	$p<0\ 01$	NS	NS	NS
Serum TSH (mU/l)					
IRDS					
Median	3 9	1 5	1 ?	1 1	0 7
Range	(<0 –11 8)	(<0 ?–6 9)	(<0 ?–5 8)	(<0 –9 0)	(<0 ?–4 6)
No	15	8	7	16	9
Controls					
Median	9 ?	3 6	? 0	1 4	1 5
Range	(<0 ?–7? 5)	(<0 ? 13 1)	(<0 –8 7)	(<0 –4 8)	(0 7–3 4)
No	5	10	17	3?	?1
	$p<0\ 01$	NS	NS	NS	NS

per liter Using routine laboratory methods the absolute concentrations of TBG TBPA and Alb in the reference serum were 9 3 mg/l 97 mg/l and 43 000 mg/l respectively

In the statistical calculations the Mann Whitney test was used (7)

RESULTS

Median body weights of the IRDS and healthy infants were comparable during the four weeks follow up (Table 1)

Median and range (lowest respective highest value) of serum T_4 serum T_3 and serum TSH concentrations are shown in Table 2

In infants with IRDS *serum levels of T_4 decreased* significantly ($p<0\ 05$) during the initial phase of the disease to minimal levels by day 3–5 whereupon a gradual increase in serum T_4 concentrations was observed (Table 2 and Fig 1) In healthy premature babies serum T_4 concentration *increased* during the early postnatal days and reached peak values by day 3–5 Serum T_4 level was lower in the sick infants compared to controls although not statistically significant for infants aged 21–30 days

Serum T_3 concentrations also *decreased* significantly ($p<0\ 02$) in infants with IRDS to low values by day 3–5 (Table 2 and Fig 1) but was followed by a rapid increase to levels

Table 1 *Median and range of body weights (gram) in relation to postnatal age in premature infants with idiopathic respiratory distress syndrome (IRDS) and in healthy controls*
No =number of samples NS=not significant

	Postnatal age in days				
	<3	3–5	6–10	11–20	20–30
IRDS (no =27)					
Median	1 915	1 950	2 000	2 240	2 340
Range	(1 055–2 700)	(1 210–2 650)	(1 155–2 300)	(1 170–2 880)	(1 240–2 690)
Controls (no =52)					
Median	2 085	2 095	2 050	2 270	2 340
Range	(850–2 800)	(850–2 615)	(1 400–2 850)	(1 170–2 960)	(1 260–3 330)
	NS	NS	NS	NS	NS

Supplementary oxygen up to an inspired oxygen concentration of 50 to 80% was adequate in 4 patients, continuous positive airway pressure (CPAP) without artificial ventilation was required in 8 infants and respirator treatment was necessary in the remaining 15 infants. Two IRDS infants with birth weights of 1 100 g and 1 560 g and gestational age of 30 weeks died by day 4 and 10 respectively. The gestational age of the surviving IRDS infants ranged from 27 to 36 weeks and birth weights ranged from 1 055–2 700 g. Gestational age was assessed as reported previously (11).

As *controls* 52 healthy prematures without perinatal asphyxia or respiratory problems were studied. Gestational age ranged from 26–36 weeks and did not differ significantly from the gestational age of IRDS infants. Birth weights ranged from 850–2 800 g and the infants were matched with the IRDS patients according to comparable body weights in all age groups (Table 1). Infants of mothers who received medication that could influence thyroid hormone or thyroid hormone binding proteins were not included.

All IRDS infants received glucose and from the third day of life also electrolytes and aminoacids through umbilical catheters. The control infants were fed with human milk. Comparable amounts of fluids were given to the IRDS and healthy prematures.

Some of the infants in both groups, particularly infants with IRDS or low birth weights, received one or more transfusions (10–15 ml/kg body weight) with packed erythrocytes during the study period. In all infants blood was collected *before* the infusion. The next sample was obtained at least 4–5 days after any transfusion at the time when pretransfusion level of thyroid hormones probably was reached (21).

Blood was drawn from a peripheral vein. One or two blood samples were obtained within the first week of life and the subsequent blood samples were collected at weekly intervals. The blood sampling was not complete because of the unfavorable conditions of the babies and the amount of serum necessary for determination of all thyroid variables (see tables). Parents were informed and consent obtained.

Serum concentrations of thyroxine (T_4) were determined using competitive binding microtechnique and serum triiodothyronine (T_3) and thyrotropin (TSH) w[...] measured by radioimmunoassay (11, 12). The thyroxi[...] binding globulin (TBG), prealbumin (TBPA) and albu[...] (Alb) were determined using rocket immunoelect[...]phoresis as previously reported (14). The thyroxi[...] binding protein concentrations were presented in terms [...] those obtained in a reference serum (100 arbitrary u[...]

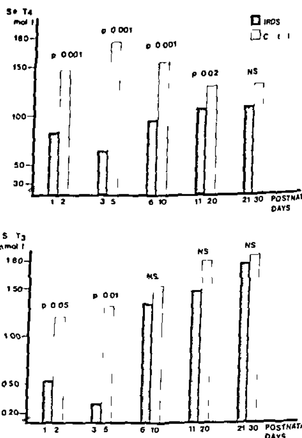


Fig. 1. Changes in serum concentrations of thyroxin[e] (T) and triiodothyronine (T_3) in premature infants wi[th] idiopathic respiratory distress syndrome (IRDS) and i[n] healthy controls. The bars indicate median values for eac[h] age group of infants. NS=not sig[nificant]

Table 3 *Median and range of serum TBG TBPA and Alb concentrations in premature infants with idiopathic respiratory distress syndrome and in controls*

No = number of samples NS = not significant

	Postnatal age in days				
	<3	3–5	6–10	11– 0	21–30
Serum TBG (arb u/l)					
IRDS					
Median	118	116	133	161	134
Range	(50–17[illegible])	(86–176)	(99–[illegible]07)	(96–198)	(105–1[illegible]6)
No	16	7	7	15	9
Control					
Median	157	166	17[illegible]	167	174
Range	(74–[illegible]34)	(1[illegible]8–[illegible]4)	(141–[illegible]9)	(90–[illegible]10)	(187–[illegible]3[illegible])
No	[illegible]1	10	11	19	15
	$p<0.01$	$p<0.01$	NS	NS	$p<0.05$
Serum TBPA (arb u/l)					
IRDS					
Median	5	30	37	44	38
Range	(15–4[illegible])	([illegible]3–36)	([illegible]1–5[illegible])	([illegible]5–59)	(34–56)
No	16	5	6	13	8
Controls					
Median	6	6	[illegible]4	30	33
Range	(10–36)	(16–37)	(19–40)	(18–47)	(11–53)
No	[illegible]0	10	9	16	13
	NS	NS	NS	$p<0.001$	NS
Serum Alb (arb u/l)					
IRDS					
Median	67	78	77	75	75
Range	(51–80)	(63–9[illegible])	(66–86)	(55–83)	([illegible]3–83)
No	16	7	6	14	9
Controls					
Median	76	75	87	75	83
Range	(5[illegible]–1[illegible]3)	(49–10[illegible])	(66–106)	(55–96)	(67–101)
No	[illegible]1	1[illegible]	11	18	14
	NS	NS	NS	NS	NS

were not significantly different in the two groups of infants (Table 3).

TBG possesses binding sites of much higher affinity for T_4 and T_3 than those of TBPA and Alb (22) and the increased serum TBG level determines the serum thyroid hormone concentrations in newborns (14). In attempting to evaluate the saturation of TBG binding sites the individual ratios between serum concentrations of thyroid hormones and TBG were calculated. Table 3 shows that median values of T_4/TBG ratios were significantly lower in infants with IRDS than in healthy infants aged 3–20 days. The T_3/TBG ratios were not significantly different in the two groups although the same tendency could be observed.

DISCUSSION

The present study demonstrates pronounced changes in serum concentrations of thyroid hormones and hormone binding proteins during the acute and recovery stages of IRDS.

The findings of low serum T_4 and serum T_3 concentrations in IRDS infants during the *first 2–3 days of life* confirm previous observa

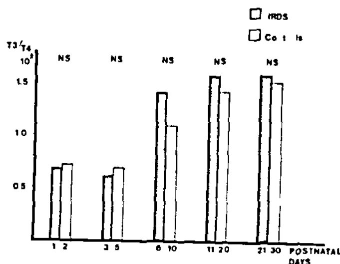


Fig 2 The ratio between serum concentrations of triiodothyronine (T_3) and thyroxine (T_4) in each age group of infants. Median values are shown. Symbols as in Fig 1

which did not differ significantly from those of healthy prematures (by day 6–10). In healthy babies a continuous rise in serum T_3 occurred during the study period. The ratio between serum T_3 and serum T_4 concentrations were not significantly different in the two groups of infants (Fig 2). The T_3/T_4 ratio increased during the second week of life and showed a tendency to higher values in infants recovering from IRDS than in healthy prematures of similar age.

Serum TSH concentrations in IRDS infants were significantly lower than in control infants during the first two days (Table 2 and Fig 3). Afterwards serum TSH values were comparable in the two groups of infants.

Median and range of thyroid hormone binding protein concentrations are presented in Table 3. *Serum TBG* values were significantly lower in infants with IRDS than in healthy prematures during the first five days of life (Fig 4) but after this an increase in serum TBG occurred. Median values of *serum TBPA* were similar in IRDS and control infants during the first week of life. In IRDS infants, however, serum TBPA increased markedly, exceeded TBPA levels in healthy prematures and reached maximum levels by 11–20 days of life (Table 3 and Fig 4). *Serum Alb* concentrations

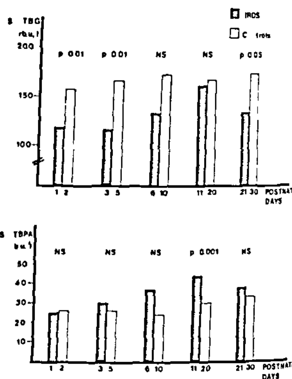


Fig 4 Changes in serum concentrations of thyroxine binding globulin (TBG) and prealbumin (TBPA) in each age group of healthy and sick premature infants. Median values are shown, symbols as in Fig 1

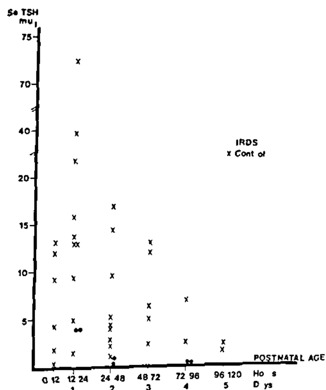


Fig 3 Serum TSH concentrations in infants with idiopathic respiratory distress syndrome (IRDS) and in healthy controls during the first five days of life. Symbols as in Fig 1

T_3 conversion in IRDS infants (1 5 17) were not confirmed by the present calculations of serum T_3/T_4 ratios in healthy and sick infants (Fig 2) but a tendency towards lower T_3/T_4 values in IRDS compared to healthy infants was observed during the first 5 days of life Serum reverse T_3 was not measured but Klein et al (17) have recently reported data indicating a decrease in serum T_3/reverse T_3 ratios in IRDS infants between 12 and 72 hours of age

Following recovery from IRDS serum T_3 concentrations increased and reached T_3 levels of healthy prematures by day 6–10 whereas the serum T_4 increase was more gradual possibly indicating an increased T_4 to T_3 conversion during the recovery stage of IRDS The T_3/T_4 ratios were similar to full terms and adults (12 18) In earlier reports on thyroid function in patients recovering from IRDS (1 6 17) the pattern of the changes in serum concentrations of T_3 and T_4 were comparable to those observed in the present study A comparison between the absolute serum concentrations would not be reasonable due to the very heterogeneous population with respect to body weights a factor known to influence the T_4 concentration in blood (7)

The serum concentrations of thyroid hormone binding proteins also changed markedly in infants during recovery from IRDS whereas only minor changes appeared in the healthy infants during the study period (Fig 4) In a report by Hardie et al (10) serum concentrations of total protein albumin and IgG globulin were found to be lower in IRDS patients than in healthy prematures

The mechanisms that determine the individual changes in serum concentrations of the thyroid hormone binding proteins are unknown but differences with respect to synthesis or utilization of the proteins are suggested The low serum TBG concentrations in IRDS infants *within* the first days of life might indicate an association between the perinatal production of pulmonal surfactant and the TBG synthesis This does not necessarily indicate a causal relationship and further investigations should be made The rapid and pronounced rise in serum TBPA in IRDS infants might possibly be caused by the increased adrenal response and higher plasma corticoid levels which have been observed in IRDS patients compared with healthy neonates (2 3) since glucocorticoids induce an increase in serum TBPA without changing serum TBG concentrations significantly (4) or reduce serum TBG binding capacity (20)

The present study provides evidence for the pronounced changes in thyroid hormones and hormone binding protein concentrations during acute and recovery stages of IRDS but the pathogenetic significance of these alterations is not clear Studies in fetal and adult animals have demonstrated the influence of thyroid hormones upon lung growth and surfactant production (8 23 29) Intraamniotic thyroid hormone application in high risk pregnancies (25) or temporary T_4 or T_3 administration to IRDS patients have been proposed (6) A supportive thyroid hormone therapy is in accordance with our suggestions of a reduced saturation of the serum thyroid hormone binding proteins Clinical trials are needed to determine whether such an early depression of thyroid function has adverse consequences Administration of thyrotropin releasing hormone (11) should also be taken into consideration

ACKNOWLEDGEMENTS

We are grateful for excellent technical assistance by the staff of the two laboratories involved Thanks to J Nyboe cand act Rigshospitalet for great help with the statistical analyses The secretarial assistance of Ms A Jacobsen is greatly appreciated

Human TSH for iodination and antihuman TSH were kindly donated by the National Pituitary Agency Bethesda USA The human TSH international reference preparation 68/38 was given by the Medical Research Council Holly Hill London

The study was supported by the Danish Medical Research Council and the Dagmar Marshall Foundation

REFERENCES

1 Abbassi V Merchant K & Abramson D Postnatal triiodothyronine concentrations in healthy pre

Table 4 *Median and range of the ratios between serum concentrations of thyroid hormone and thyroxine binding globulin (TBG) in infants with idiopathic respiratory distress syndrome (IRDS) and in healthy controls*

No = number of samples NS = not significant

	Postnatal age in days				
	<3	3–5	6–10	11–20	21–30
T_3/TBG ×10^3					
IRDS					
Median	50	38	62	98	145
Range	(13–152)	(31–45)	(51–103)	(54–141)	(61–179)
No	13	2	6	11	9
Controls					
Median	62	70	78	115	116
Range	(16–112)	(40–82)	(61–150)	(77–150)	(86–162)
No	19	5	7	16	14
	NS	NS	NS	NS	NS
T_4/TBG ×10^2					
IRDS					
Median	79	57	50	65	76
Range	(47–147)	(16–68)	(43–76)	(34–141)	(42–107)
No	16	7	7	15	9
Controls					
Median	86	95	101	87	75
Range	(50–151)	(58–132)	(48–131)	(46–126)	(53–127)
No	21	10	11	19	15
	NS	$p<0.001$	$p<0.001$	$p<0.01$	NS

tions (1, 5, 6, 17, 24). In addition the study provides evidence that a *decrease* in thyroid hormone concentrations occurs during the acute stage of IRDS. This is in contrast to the early postnatal *rise* in thyroid hormone serum levels which appears in healthy babies due to the TSH surge (9, 12). In IRDS infants the TSH stimulation of the thyroid gland is probably less pronounced since the serum TSH levels during the first days after birth were significantly lower than in healthy prematures. Similar TSH findings have recently been reported by Cuestas & Engel (6) and Klein et al. (17). Later in infancy serum TSH concentrations were similar in the two groups of infants and therefore cannot explain the ensuing changes in serum T_4 and T_3 concentrations.

The serum TBG concentrations were significantly lower in infants during the acute stage of IRDS than in healthy prematures, whereas serum TBPA and Alb levels were comparable in the two groups of infants, confirming our preliminary results (13, 15). The low serum TBG level contributes to explanation of the diminished serum thyroid hormone concentrations in IRDS patients. Calculations of the ratio between serum concentrations of thyroid hormones and TBG also indicate more unsaturated binding sites upon TBG in IRDS infants than in healthy prematures (Table 4). Redding & Pereira (24) and Cuestas & Engel (6) reached the same conclusion by evaluating the free thyroxine index. It is not known whether a reduced thyroid hormone secretion or an increased utilization of T_4 and T_3 occur during the initial phase of IRDS. Findings of low oxygen consumption in IRDS infants compared to healthy prematures (19) agree with the hypothesis of a decrease in the activity of the thyroid gland.

Previous suggestions of a decreased T_4 to

THE EFFECT OF FEEDS OF DIFFERING COMPOSITION ON ENTERO INSULAR HORMONE SECRETION IN THE FIRST HOURS OF LIFE IN HUMAN NEONATES

A AYNSLEY-GREEN A LUCAS and S R BLOOM

From the University Department of Paediatrics Oxford and the Hammersmith Hospital London Great Britain

ABSTRACT Aynsley Green A Lucas A and Bloom S R (University Department of Paediatrics Oxford and Hammersmith Hospital London Great Britain) The effect of feeds of different composition on entero insular hormone secretion in the first hours of life in human neonates Acta Paediatr Scand 68 265 1979.—Little is known on the enteral stimuli for gastro-intestinal hormone release in newborn infants We have compared the effect of the first feed of human breast milk (5 ml/kg) or 10% dextrose (5 ml/kg) on blood glucose and plasma gastrin enteroglucagon Gastric Inhibitory polypeptide (GIP) pancreatic glucagon and insulin in 21 full term infants at 4–6 hours of age The first feed of human milk caused a rise in blood glucose and plasma insulin gastrin and enteroglucagon but no change occurred in GIP or pancreatic glucagon The 10% dextrose feed did not stimulate enteroglucagon release although similar changes occurred in blood glucose and plasma insulin and gastrin We conclude that the composition of the feed influences the pattern of gastro-intestinal hormone release during the first hours of life and that the entero-insular responses to feeding differ in the neonate and the adult

KEY WORDS Human milk dextrose glucose insulin glucagon enteroglucagon gastrin gastric inhibitory peptide newborn

In recent years the gut has emerged as an important endocrine organ producing a series of gastro intestinal hormones (18) The development of radioimmunoassay techniques for the measurement of these hormones in plasma has permitted *in vivo* physiological studies (3) In the neonate the physiological release of gut hormones in response to feeding may play an important role in the adaptation to extra uterine nutrition (vide infra) However although some gastro intestinal hormones have been shown to be present in the foetal gut (13 17) there have been few studies demonstrating the physiological stimuli for gut hormone release in the newborn infant

As part of a previous study we examined the effect of the first feed of breast milk on the release of gastrin enteroglucagon and gastric inhibitory polypeptide (GIP) together with the effects on plasma insulin and on blood glucose (2) In the present study we compare these effects of the first milk feeding with those of a first enteral feeding of 10% dextrose

METHODS

Twenty-one infants at or near to term were studied with the approval of the ethics committee All had been admitted to the Special Care Baby Unit and all had indwelling umbilical arterial catheters for clinical monitoring purposes They were nursed in incubators at an environmental temperature appropriate for their weight and gestation None had a rectal temperature of <36 none had a pH <7 25 P_{O_2} was maintained in the range 8–12 kP (60–90 mmHg) and the clinical condition of the infants was stable throughout the study Infants were randomly allocated into two groups

Group I comprised 9 infants who received a first feed of 10% dextrose (*vide infra*) Their mean birth weight was 2 570 g (range 2 070–3 090 g) and mean gestational age 36 9 weeks (range 36–40 weeks) Five had mild to moderate respiratory distress 3 of whom required additional inspired oxygen The other 4 had moderate birth asphyxia requiring transient endotracheal intubation oxygen and alkali therapy

Group II comprised 1 infants who received a first feed of human milk (*vide infra*) Their mean birthweight was

term infants and in infants with respiratory distress syndrome *Pediatr Res 11* 802 1977
2 Bacon G F George R Koeff S T & Howatt W F *Plasma corticoids in the respiratory distress syndrome in normal infants Pediatrics 55* 500 1975
3 Ballard R A & Ballard P L Use of prenatal glucocorticoid therapy to prevent respiratory distress syndrome *Am J Dis Child 130* 982 1976
4 Burr W A Ramsden D B Griffiths R S Black E G & Hoffenberg R Meinhold H & Wenzel K W Effect of a single dose of dexamethasone on serum concentrations of thyroid hormones *Lancet II* 58 1976
5 Cuestas R A Lindall A & Engel R R Low thyroid hormones and respiratory distress syndrome of the newborn Studies on cord blood *N Engl J Med 295* 297 1976
6 *Cuestas R A & Engel R R Low thyroid activity* in prematures with RDS (Abstract) *Pediatr Res 11* 474 1977
7 Dussault J H Morissette J Fiset P Laberge E & Laberge C Factors influencing results for thyroxine concentration in blood as measured in paper filter spots in a screening program for neonatal hypothyroidism *Clin Chem 22* 1392 1976
8 Erenberg A Omori K Menkes J H Oh W & Fisher D A Growth and development of the thyroidectomized ovine fetus *Pediatr Res 8* 783 1974
9 Erenberg A Phelps D L Lam R & Fisher D A Total and free thyroid hormone concentrations in the neonatal period *Pediatrics 53* 211 1974
10 Hardie G Harrison V C & Kench J E Further observations on serum proteins in respiratory distress syndrome of the newborn *Arch Dis Child 43* 471 1968
11 Jacobsen B B Andersen H J Dige Petersen H & Hummer L Pituitary thyroid responsiveness to thyrotropin releasing hormone in preterm and small *for gestational age newborns Acta Paediatr Scand 66* 541 1977
12 Jacobsen B B Andersen H J Peitersen B Dige Petersen H & Hummer L Serum levels of thyrotropin thyroxine and triiodothyronine in fullterm small for gestational age and preterm newborn babies *Acta Paediatr Scand 66* 681 1977
13 Jacobsen B B Peitersen B & Andersen H J Serum levels of thyroxine binding proteins in infants with respiratory distress syndrome *The 16th annual meeting of the European Society for Pediatric Endocrinology* Session for recent results Cambridge 1977
14 Jacobsen B B Peitersen B Andersen H J & Hummer L *Serum concentrations of thyroxine* binding globulin prealbumin and albumin in healthy fullterm small for gestational age and preterm newborn infants *Acta Paediatr Scand 68* 49 1979
15 Jacobsen B B & Peitersen B Comparisons between serum concentrations of thyroxine and thyroxine binding proteins in samples simultaneously obtained from capillary peripheral vein central vein *and aorta in newborn infants Acta Paediatr Scand 68* 43 1979
16 Kamper J Bækgaard P Peitersen B Christiansen P M Tygstrup I & Friis Hansen B Behandling af respiratory distress syndrome med overtryk i luftvejene (continuous positive airway pressure) *Ugeskr Laeger 135* 2207 1973
17 Klein A H Foely B Ho R S Kenny F M & Fisher D A Thyroid response to partuntion Relationship to gestational age and the respiratory distress syndrome (Abstract) *Pediatr Res 11* 477 1977
18 Klein A H Stinson D Foley B Larsen P R & Foley T P Thyroid function studies in preterm infants recovering from the respiratory distress syndrome *J Pediatr 91* 261 1977
19 Levison H Deli Papadoulos M & Swyer P R Oxygen consumption in newly born infants with the respiratory distress syndrome *Biol Neonate 7* ?– 1964
20 *Oppenheimer J H & Werner S Effect of prednisone* on thyroxine binding proteins *J Clin Endocrinol 26* 715 1966
21 Paletta B Rossipal E & Haidvogl M Veränderung des PBI Spiegels bei Neugeborenen nach Austauschtransfusion *Padiatr Padol 5* 339 1969
22 Prince H P & Ramsden D B A new theoretical description of the binding of thyroid hormones by serum proteins *Clin Endocrinol 7* 307 1977
23 Redding R A & Douglas W H J Thyroid hormone influence upon lung surfactant metabolism *Science 175* 994 1972
24 Redding R A & Pereira C Thyroid function in respiratory distress syndrome of the newborn *Pediatrics 54* 423 1974
25 Sack J Mashiachi S Barkai G Lunenfeld B Brish M Goldman B & Serr D M Intraamniotic thyroxine effect in high risk pregnancies *The 16th annual meeting of the European Society for Pediatric Endocrinology* Cambridge 1977
26 Schaffer A J & Avery M E *Diseases of the newborn Saunders Philadelphia* London and Toronto 1977 4th ed p 134
27 Siegel S *Nonparametric statistics for the behavioral sciences* McGraw Hill Kogakusha Tokyo 1956
28 Smith D W Klein A M Henderson J R & *Myrianthopoulos N C Congenital hypothyroidism* signs and symptoms in the newborn period *J Pediatr 87* 958 1975
29 Wu B Kikkawa Y Orzalesi M M Motoyama E K Kaibara M Zigas C J & Cook C D The effect of thyroxine on the maturation of fetal rabbit lungs *Biol Neonate 22* 161 1973

Submitted June 3 1978
Accepted July 24 1978

(B B J) The University Clinic of Paediatrics
Children s Hospital Fuglebakken
Drosselvej 57
2000 Copenhagen F
Denmark

Acta Pædiatr Scand 68 265–270 1979

THE EFFECT OF FEEDS OF DIFFERING COMPOSITION ON ENTERO INSULAR HORMONE SECRETION IN THE FIRST HOURS OF LIFE IN HUMAN NEONATES

A AYNSLEY-GREEN A LUCAS and S R BLOOM

From the University Department of Paediatrics Oxford and the Hammersmith Hospital London Great Britain

ABSTRACT Aynsley Green A Lucas A and Bloom S R (University Department of Paediatrics Oxford and Hammersmith Hospital London Great Britain) The effect of feeds of different composition on entero insular hormone secretion in the first hours of life in human neonates Acta Paediatr Scand 68 265 1979.—Little is known on the enteral stimuli for gastro-intestinal hormone release in newborn infants We have compared the effect of the first feed of human breast milk (5 ml/kg) or 10% dextrose (5 ml/kg) on blood glucose and plasma gastrin enteroglucagon Gastric Inhibitory polypeptide (GIP) pancreatic glucagon and insulin in 21 full term infants at 4–6 hours of age The first feed of human milk caused a rise in blood glucose and plasma insulin gastrin and enteroglucagon but no change occurred in GIP or pancreatic glucagon The 10% dextrose feed did not stimulate enteroglucagon release although similar changes occurred in blood glucose and plasma insulin and gastrin We conclude that the composition of the feed influences the pattern of gastro-intestinal hormone release during the first hours of life and that the entero-insular responses to feeding differ in the neonate and the adult

KEY WORDS Human milk dextrose glucose insulin glucagon enteroglucagon gastrin gastric inhibitory peptide newborn

In recent years the gut has emerged as an important endocrine organ producing a series of gastro intestinal hormones (18) The development of radioimmunoassay techniques for the measurement of these hormones in plasma has permitted *in vivo* physiological studies (3) In the neonate the physiological release of gut hormones in response to feeding may play an important role in the adaptation to extra uterine nutrition (vide infra) However although some gastro intestinal hormones have been shown to be present in the foetal gut (13 17) there have been few studies demonstrating the physiological stimuli for gut hormone release in the newborn infant

As part of a previous study we examined the effect of the first feed of breast milk on the release of gastrin enteroglucagon and gastric inhibitory polypeptide (GIP) together with the effects on plasma insulin and on blood glucose (2) In the present study we compare these effects of the first milk feeding with those of a first enteral feeding of 10% dextrose

METHODS

Twenty-one infants at or near to term were studied with the approval of the ethics committee All had been admitted to the Special Care Baby Unit and all had indwelling umbilical arterial catheters for clinical monitoring purposes They were nursed in incubators at an environmental temperature appropriate for their weight and gestation None had a rectal temperature of <36 none had a pH <7 25 P was maintained in the range 8–12 kP (60–90 mmHg) and the clinical condition of the infants was stable throughout the study Infants were randomly allocated into two groups

Group I comprised 9 infants who received a first feed of 10% dextrose (*vide infra*) Their mean birth weight was 2570 g (range 2070–3 090 g) and mean gestational age 36 9 weeks (range 36–40 weeks) Five had mild to moderate respiratory distress 3 of whom required additional inspired oxygen The other 4 had moderate birth asphyxia requiring transient endotracheal intubation oxygen and alkali therapy

Group II comprised 12 infants who received a first feed of human milk (*vide infra*) Their mean birthweight was

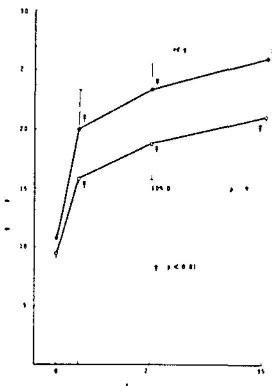


Fig 1 The effect of the first feed of 10% dextrose or breast milk on plasma gastrin (±S E M)

2945 (range 2 110–3 940) and a mean gestational age of 37 8 weeks (range 37–39 weeks) Nine infants were suffering from moderately severe respiratory distress requiring enriched inspired oxygen Of the other 3 one was a breech delivery with severe birth asphyxia and 2 had lesser degrees of birth asphyxia requiring transient endotracheal intubation oxygen and alkali therapy

Feeding procedure

The first feed was given between 4 and 6 hours of age via a nasogastric tube which was passed at least 2 hours before feeding Group I received a 5 ml/kg bolus of 10% dextrose Group II a 5 ml/kg bolus of breast milk This volume gives 60 ml/kg per 24 hours with a 2 hourly feeding regime The feed was given by gravity over 2–4 min with the infant lying on the right side with the head of the mattress raised *Infants were not disturbed during the study period* At the end of the test the stomach contents were re aspirated and in no case was more than 25% of the original feed volume returned Regurgitation of the feed did not occur in any instance

Blood sampling and assay methods

Blood samples were drawn from an indwelling umbilical artery catheter immediately before the feed and at 5 25 and 55 min after the feed Samples were analysed for plasma gastrin pancreatic glucagon enteroglucagon gastric inhibitory polypeptide (GIP) and insulin and for blood glucose The details of the sampling procedure and of the assay methods used are described in our previous publication (2) Results were assessed for significance by means of Student s *t* test assuming equal variance

RESULTS

Gut hormones

In the 10% dextrose fed group the mean basal plasma gastrin level was 9 4±2 2 (S E M) pmol/l rising immediately and progressively to 21±3 7 pmol/litre at 55 min following the feed ($p<0.01$) and in the milk fed group gastrin rose from a fasting level of 10 9±1 pmol/l to 26 1±1 9 pmol/l at 55 minutes ($p<0.01$) There were no significant differences between the mean gastrin level in the two groups either before or at 5 25 or 55 min after the feed (Fig 1)

The mean basal plasma GIP level was 126±27 (S E M) pg/ml in the 10% dextrose fed group and 118±32 pg/ml in the milk fed group In neither group was there a significant change in level after feeding (Table 1)

Mean basal enteroglucagon level was 139±16 (S E M) pg/ml equivalent in the 10% dextrose fed group and there was no significant change with feeding In contrast in the milk fed group there was a significant rise in enteroglucagon level from 145±30 pg/ml equivalent to 306±65 at 55 min ($p<0.05$) which was

Fig 1 *Effect of the first feed of 10% dextrose compared with breast milk on plasma gastric inhibitory polypeptide (pg/ml ± S E M)*

Time (min)	0	5	25	55
10% dextrose fed group $n=5$	126±27	165±18 N S	186±43 N S	170±25 N S
Milk fed group $n=8$	118±32	203±79 N S	169±38 N S	154±28 N S

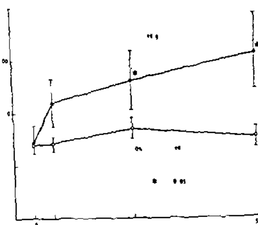


Fig 2 The effect of the first feed of 10% dextrose or breast milk on plasma enteroglucagon (±S E M)

significantly higher than the level attained at 55 min in the 10% dextrose fed group ($p<0$ 05) (Fig 2) Mean basal plasma pancreatic glucagon was 57±12 pmol/l in the 10% dextrose group and 65±12 pmol/l in the milk fed group There was no change in level in either group following the feed and there were no differences in levels between the two groups (Table 2)

Plasma insulin and blood glucose

The mean basal plasma insulin level in the 10% dextrose fed group was 5 1±1 2 (S E M) mU/l and a significant rise occurred following the feed reaching 11 0±1 7 mU/l at 55 min ($p<0$ 01) In the milk fed group basal plasma insulin was 6 8±1 3 mU/l rising to 15 3±3 2 at 55 min ($p<0$ 01) There were no significant differences between the two groups in mean insulin levels before and at 5 25 and 55 *min* after the feed (Fig 3)

Mean basal blood glucose concentration in the 10% dextrose fed group was 3 73±0 22 mmol/l rising to 4 93±0 56 at 55 *min* ($p<$ 0 01) In the milk fed group mean basal blood glucose was 3 67±0 22 *mmol/l* rising to 4 51± 0 25 at 55 min ($p<0$ 01) The glycaemic responses in the two groups did not differ significantly (Fig 4)

DISCUSSION

After birth enteral feeding triggers several important structural and physiological developmental changes in the neonate which may be considered to be a part of his adaptation to extra uterine nutrition In piglets (20 23) and rats (14) feeding results in marked structural changes and growth of the digestive tract and in piglets (23) jejunal lactase and acid phosphatase activities are enhanced In addition it has been demonstrated that early feeding increases the responsiveness to glucose of pancreatic β cells in neonatal rats (1) and piglets (7) It is suggested that the intermediary link between feeding and the changes described above may be in part the release of gut hormones In support of this hypothesis is the trophic action of some gut hormones in adult animals and man gastrin (10 11) and enteroglucagon (8) stimulate structural development of the gut and cholecystokinin (15) and gastrin (16) stimulate the growth of the exocrine pancreas Moreover Lichtenberger (14) has shown in neonatal rats that feeding produces rises in plasma gastrin which parallel the induced gastro intestinal changes Plasma

Table 2 *Effect of the first feed of 10% dextrose compared with breast milk on plasma pancreatic glucagon (pmol/l ± S E M)*

Time (min)	0	5	25	55
10% dextrose fed group $n=9$	57±12	68±15	63±12	63±11
Milk fed group $n=11$	65±12	68±12	73±13	67±13

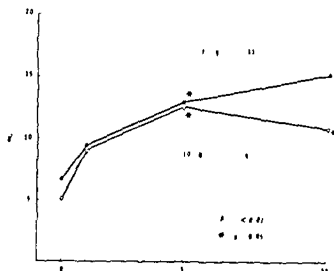


Fig 3 The effect of the first feed of 10% dextrose or breast milk on plasma insulin (±S E M)

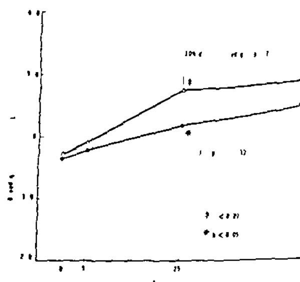


Fig 4 The effect of the first feed of 10% dextrose or breast milk on blood glucose (±S E M)

gastrin together with enteroglucagon are known to increase during the first 4 days of life in the human neonate (19) Von Berger (22) has shown that gastrin increases following the first milk feeding however the effect of feeding on other gut hormones in the neonate has received little attention In the present study we have examined the effect of two enteral stimuli, 10% dextrose and human milk given to previously unfed sick neonates on the release of three gut hormones (gastrin enteroglucagon and GIP) together with the effects on plasma insulin and on blood glucose

The gastrin responses to the first feed of human milk and of 10% dextrose were similar with a significant rise at 5 25 and 55 min following the feed In adults digested protein is a potent stimulus for gastrin release it is therefore interesting that milk feeding did not produce a significantly greater response than 10% dextrose feeding This could be explained by the absence of sufficient proteolysis during the 55 min study period following the feed or might be due to a failure of neonatal gastrin secreting cells to respond to the products of protein digestion Gastric distension may well have been the main stimulus to gastrin release in this study

Enteroglucagon levels were significantly raised at 25 and 55 min following the first feed of human milk though the fasting level was unchanged by a 10% dextrose feed In adults enteroglucagon release is strongly stimulated by carbohydrate and moderately stimulated by long chain triglycerides (9) It was therefore surprising that there was no enteroglucagon response to a 10% dextrose feed but this may be because glucose is rapidly absorbed in the upper small intestine whereas enteroglucagon is mostly found in the ileum It is also possible that in the neonate triglyceride is a more potent stimulus to enteroglucagon release than in the adult perhaps as an adaptation to the high fat intake of the milk fed infant

Gastric inhibitory polypeptide did not increase significantly after a milk or 10% dextrose feed though in both cases post prandial levels tended to be higher than fasting levels In adults the GIP response to feeding is biphasic with an early peak at 45 min in response to carbohydrate followed by a late plateau at 2–3 hours in response to fat (4) Since our postprandial study period lasted only 55 min we might not have seen a delayed fat response We failed to achieve a significant early GIP rise even with 10% dextrose which has a higher carbohydrate content than milk GIP is known to release insulin in adults (5) and King et al (12) have speculated that their failure to enhance the insulin response to enteral

compared with parenteral glucose in neonates was due to a failure of responsiveness of neonatal pancreatic B cells to GIP In view of our findings we would like to propose an alternative hypothesis that the failure of gastrointestinal enhancement of the insulin response to glucose is due to a failure of early feeding to release GIP and it is possible that the increasing glucose response to insulin induced by later feeds (1 7) is due to the initiation of GIP release

The glycaemic response to 10% dextrose and milk feeding was not significantly different in spite of the high carbohydrate content of the former The insulin response to 10% dextrose appeared less than that to a milk feed but this difference was not significant Gastrin (6) enteroglucagon (21) and GIP (5) may all be stimuli to insulin release in adults In our study gastrin and GIP responses were similar in milk fed and 10% glucose fed neonates whereas enteroglucagon was stimulated only by milk feeding If enteroglucagon were a potent stimulus of insulin release in the neonate a greater insulinaemic response would be expected in the milk fed group There was in fact a higher insulin glucose ratio in the milk fed group 55 min after feeding (mean ratio 3 8) than in the 10% dextrose fed group (mean ratio 2 1) However since this difference was not significant we have failed to demonstrate that enteroglucagon is an important effector in the entero insular axis in newborn infants

It is concluded that gut hormone release shortly after birth may differ from that in adults not only in the quantity of response evoked by an enteral stimulus but in the quality of stimulus required to produce a response However it is possible that these differences may relate to the fact that our studies were confined to sick neonates

Further work is needed to define the gut hormone responsiveness of well and ill preterm and full term neonates at different postnatal ages fed in different ways since this information may provide a rational basis for designing optimal feeding regimes which will stimulate most effectively high risk neonates in their physiological adaptation to feeding

ACKNOWLEDGEMENTS

We thank Professor J P M Tizard for his advice and encouragement the staff of the Special Care Baby Unit for their help and Dr D H Williamson and Dr R C Turner for their assistance with glucose and insulin assays

REFERENCES

1 Asplund K Effects of postnatal feeding on the functional maturation of pancreatic islet B cells of neonatal rats *Diabetologia* 8 153 1972
2 Aynsley Green A Bloom S R Williamson D H & Turner R C Endocrine and metabolic response in the human newborn to first feed of breast milk *Arch Dis Child* 52 4 ?91 1977
3 Bloom S R Radioimmunoassay of intestinal hormones *Gut* 15 50? 1974
4 Brown J C Dryburgh J R Frost J L Otte S C & Pederson R A Properties and actions of GIP *In* S R Bloom (ed) *Gut Hormones* Churchill Livingstone Edinburgh 1978 pp ?77–?8?
5 Dupre J Ross S A Watson D & Brown J C Stimulation of insulin secretion by gastric inhibitory polypeptide in man *J Clin Endocrinol Metab* 37 8?6 1973
6 Dupre J Curtis J D Unger R H Waddell R W & Beck J C Effects of secretin pancreozymin or gastrin on the response of the endocrine pancreas to administration of glucose of arginine in man *J Clin Invest* 48 745 1969
7 Gentz J Persson B Kellum M Bengtsson G & Thorell J Effect of feeding on intravenous glucose tolerance and insulin response in piglets during the first day of life *Life Science* 10 2 137 1971
8 Gleeson M H Bloom S R Polak J M Henry K & Dowling R M An endocrine tumour in kidney affecting small bowel structure motility and absorptive function *Gut* 12 733 1971
9 Holst J J Christiansen J & Kuhl C The enteroglucagon response to intrajejunal infusion of glucose triglycerides and sodium chloride *Scand J Gastroenterol* 11 ?97 1976
10 Johnson L R The trophic action of gastrointestinal hormones *Gastroenterology* 70 278 1976
11 Johnson L R New aspects of the trophic action of gastrointestinal hormones *Gastroenterology* 7? 88 1977
1? King K C Schwartz R Yamaguchi K & Adam P A J Lack of gastrointestinal enhancement of the insulin response to glucose in newborn infants *J Paediatr* cs 91 5 783 1977
13 Larsson L I Rehfeld J F & Goltermann N Gastrin in the human fetus *Scand J Gastroenterol* 12 869 1977
14 Lichtenberger L & Johnson L R Gastrin in the

ontogenic development of the small intestine *Am J Physiol 227* 2 390 1974

15 Mainz D L Black O & Webster P D *Hormonal* control of pancreatic growth *J Clin Invest 52* 2300 1973

16 Mayston P D & Barrowman J A The influence of chronic administration of pentagastrin on the rat pancreas *Am J Exp Physiol 56* 113 1971

17 Moxey P C & Trier J S Development of endocrine cells in the human fetal small intestine *Gastroenterology 72* 4 p 819 (abstracted) 1977

18 Pearse A G E Polak J M & Bloom S R The newer gut hormones *Gastroenterology 72* 746 1977

19 Rogers I M Davidson D C Lawrence J Ardill J & Buchanan K D Neonatal secretion of gastrin and glucagon *Arch Dis Child 49* 796 1974

20 Stoddart R W & Widdowson E M Changes in the organs of pigs in response to feeding for the first 24 hours after birth III Fluorescence Histochemistry of the Carbohydrates of the Intestine

21 Unger R H Ohneda A Valverde I Eisentraut A M & Exton J Characterisation of the responses of circulating glucagon like immunoreactivity to intraduodenal and intravenous administration of glucose *J Clin Invest 47* 48 1968

22 Von Berger L Henrichs I Raptis S Heinze E Jonathan W Teller W M & Pfeiffer E F Gastrin concentration in plasma of the neonate at birth and after the first feeding *Paediatrics* 58 264 1976

23 Widdowson E M Colombo V E & Artavanis C A Changes in the organs of pigs in response to feeding for the first 24 hours after birth II The digestive tract *Biol Neonate* 28 272 1976

Submitted July 17 1978
Accepted Oct 10 1978

(A A G) University Department of Paediatrics
John Radcliffe Hospital
Headington
Oxford OX3 9DU
U K

Acta Pædiatr Scand 68 271–272 1979

SHORT COMMUNICATION

FAECAL LEUCOCYTES IN CAMPYLOBACTER ASSOCIATED DIARRHOEA IN INFANTS

Campylobacters have recently been recognized as important causative agents of human diarrhoea in all age groups (1) In the study of Skirrow (5) campylobacters were found to account for 7 1% of all unselected cases of diarrhoea The clinical picture varies but abdominal pains and the presence of blood pus and mucus in the stools have been reported These features are typical of exudative diarrhoea where polymorphonuclear leucocytes (PMNL) are often found in the faeces

We have attempted to isolate campylobacters from all patients admitted for diarrhoea to the Department of Paediatrics at the Tampere Central Hospital over a period of 5 months beginning in May 1978 At the same time we have looked for PMNL in the stools The preparation for PMNL was made by smearing mucus from a fresh stool specimen onto a glass slide which was dried in the air stained and examined microscopically (Fig 1) The culture method for campylobacters was adapted from Skirrow (5) and Lauwers et al (3)

Campylobacters were isolated in 5 cases during the 5 month period among 141 patients hospitalized for diarrhoea (3 5%) Faecal leucocytes were demonstrated in 20 cases (14 2%) Of the 5 campylobacter positive patients PMNL were found in the faeces of 4 cases Therefore campylobacters accounted for 20% of all cases of exudative diarrhoea in this series In addition we saw a case of campylobacteriosis in a 9-day old infant with PMNL in the faeces before the systematic screening for campylobacters was begun We have included this case in Table 1

The main clinical characteristics of campylobacter associated disease in the 6 infants are presented in Table 1 The diarrhoea was exudative in 5 cases with either gross blood or mucus or both present With the exception of Case 1 (9 day old infant) all the other patients remained in good condition despite prolonged diarrhoea Their routine laboratory tests remained unremarkable In no case was there vomiting associated with diarrhoea but at least two of the patients experienced abdominal pains

Faecal leucocytes are seen in exudative diarrhoea which usually is caused by invasive intestinal pathogens i e *Salmonella Shigella* enteroinvasive strains of *E Coli* (2) and *Yer*

Table 1 *Clinical characteristics of six cases of campylobacter associated diarrhoea in infants*

Case	Age	Duration of symptoms (days)	Fever (°C)	WBC	Quality of stools	PMNL in stools
1	9 days	3	39 6	15 400	Mucoid	+
	[illegible] months	11	37 6	11 400	Mucoid bloody	+
3	1[illegible] months	9	38 4	7 900	Watery	–
4	14 months	10	38 3	12 800	Mucoid bloody	+
5	1 months	8	40 0	8 [illegible]00	Mucoid bloody	+
6	4 months	[illegible]0	37 8	4 400	Watery bloody	+

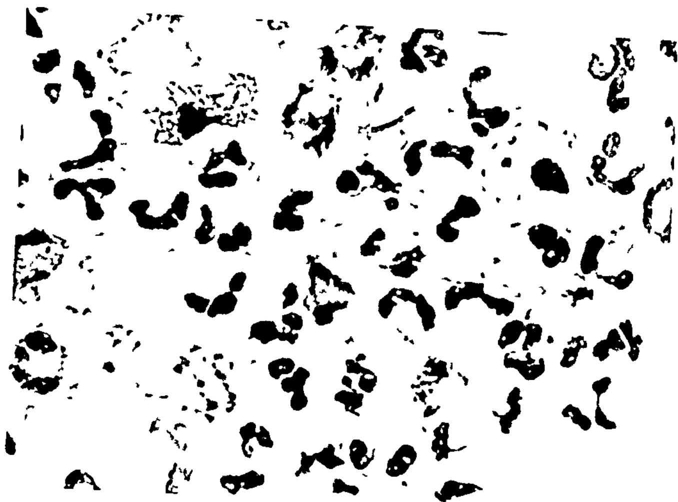


Fig. 1. Faecal leucocytes from a case of campylobacter associated diarrhoea. Mucus from the stools was smeared on a glass slide, dried and stained with Gram stain. ×1 000.

sinia enterocolitica (4). We also looked for these bacteria in the present patients with negative results. It now seems that campylobacters may be an important cause of exudative diarrhoea in our community. Campylobacteriosis appeared to be endemic, as there was no history of travelling abroad in these patients. In any case, campylobacters must be sought along with other intestinal pathogens in exudative diarrhoea of infants.

Markku Mäki, Ritva Mäki
Timo Vesikari

Departments of Paediatrics and Microbiology
Tampere Central Hospital
33250 Tampere 52
Finland

REFERENCES

1. Editorial: Campylobacter enteritis. *Lancet II*: 135, 1978.
2. Harris, J. C., DuPont, H. L. & Hornick, R. B.: Fecal leukocytes in diarrheal illness. *Ann Intern Med 76*: 697, 1972.
3. Lauwers, S., DeBoeck, M. & Butzler, J. P.: Campylobacter enteritis in Brussels. *Lancet I*: 604, 1978.
4. Mäki, M., Grönroos, P. & Vesikari, T.: In vitro invasiveness of Yersinia enterocolitica isolated from children with diarrhea. *J Infect Dis*, in press.
5. Skirrow, M. B.: Campylobacter enteritis: a new disease. *Br Med J II*: 9, 1977.

Acta Pædiatr Scand 68 273–274 1979

SHORT COMMUNICATION

LYSOZYME AND COMPLEMENT FACTORS IN SERA FROM CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA

It has been suggested that serial determinations of serum lysozyme may be of value in detecting early systemic relapses of acute lymphoblastic leukemia (ALL) (4 7) This supposition is based on the finding that the concentration of serum lysozyme in children with ALL fluctuates with the course of the disease being low before treatment and during relapse (4 5 7) Similarly high serum concentrations of the C1 esterase inactivator (C1 IA) and of the complement factor C4 are found in patients with malignant diseases including leukemia with a tendency to normalization during successful cytostatic treatment or after surgical removal of the cancer (2) Thus in malignancies the serum levels of C1 IA and of C4 apparently parallel disease activity (1 2)

In order to assess whether changes in the serum concentrations of lysozyme and of the complement factors C1 IA C3 and C4 could warn about impending relapse in ALL we have made serial determinations of these serum components in 30 children with ALL age 2–14 years All children received conventional cytostatic treatment (6) Serum lysozyme concentration was determined by a nephelometric method (3) Serum C1 IA C3 and C4 were determined by single radial immunodiffusion

Low serum lysozyme concentrations were found in children with untreated ALL and in children with hematological relapses of the disease as other workers have reported (4 5) The mean serum lysozyme value in these patients were 2 14 mg/l (ranges 5–6 5 mg/l) These values were significantly different ($p<0\ 05$) from those found in patients in remission on treatment mean 4 13 mg/l (ranges 1 0–0 5 mg/l) off treatment mean 4 60 mg/l (ranges 0–10 0 mg/l) and in healthy controls mean 4 33 mg/l (ranges ? 57–7 0 mg/l) Five patients relapsed during the observation period Their serum lysozyme values prior to during and following relapses are given in Fig 1 Three of the five patients had abnormally low serum lysozyme values at the time of relapse but only one of the five patients had low values 1 month prior to the relapse In agreement with reports from Finch et al (5) we found abnormally low lysozyme concentrations in sera from patients in remission with signs of bone marrow hypoplasia secondary to cytostatic treatment

The mean serum concentration of C1 IA in children with ALL before treatment or in relapse was 0 48 g/l (Fig 2) In children in remission either on or off treatment the mean serum concentration of C1 IA was 0 38 g/l (Fig 2) The corresponding

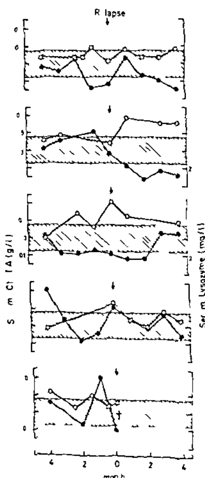


Fig 1 The serum concentrations of lysozyme (●) and C1-esterase inactivator (O) in 5 children with acute lymphoblastic leukemia before during and following bone marrow relapse (indicated by arrows) One of the patients died (†) shortly after the relapse had been diagnosed Shaded area = reference ranges

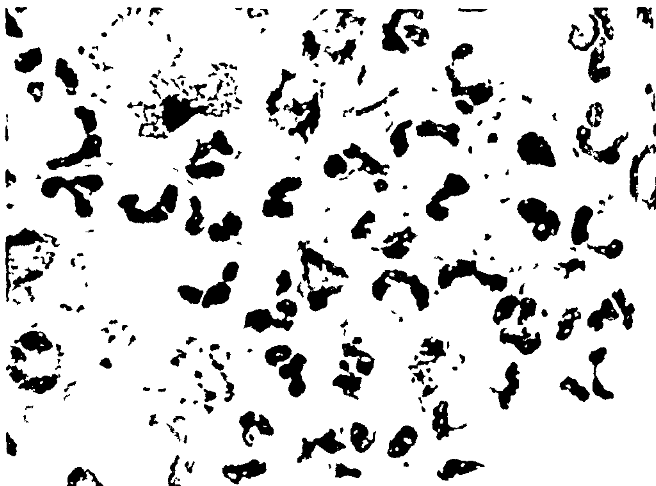


Fig 1 Faecal leucocytes from a case of campylobacter associated diarrhoea Mucus from the stools was smeared on a glass slide dried and stained with Gram stain ×1 000

sinia enterocolitica (4) We also looked for these bacteria in the present patients with negative results It now seems that campylo bacters may be an important cause of exuda tive diarrhoea in our community Campylo bacteriosis appeared to be endemic as there was no history of travelling abroad in these pa tients In any case campylobacters must be sought along with other intestinal pathogens in exudative diarrhoea of infants

Markku Maki Riita Maki
Timo Vesikari
Departments of Paediatrics and Microbiology
Tampere Central Hospital
33250 Tampere 52
Finland

REFERENCES

1 Editorial Campylobacter enteritis *Lancet II* 135 1978
2 Harris J C DuPont H L & Hornick R B Fecal leukocytes in diarrheal illness *Ann Intern Med* 76 697 1972
3 Lauwers S DeBock M & Butzler J P Campylo bacter enteritis in Brussels *Lancet I* 604 1978
4 Maki M Gronroos P & Vesikari T In vitro inva siveness of Yersinia enterocolitica isolated from chil dren with diarrhea *J Infect Dis* in press
5 Skirrow M B Campylobacter enteritis a new dis ease *Br Med J II* 9 1977

Acta Pædiatr Scand 68 275–276 1979

SHORT COMMUNICATION

EXOCRINE PANCREATIC INSUFFICIENCY SMALL INTESTINAL DYSFUNCTION AND PROTEIN INTOLERANCE

A Chance Occurrence or a Connection?

The coexistence of cystic fibrosis (CF) and coeliac disease (CD) is reported in eight children (for ref see 4 5) the odds against this occurring in the same child being 1 2 million to 1 5 9 million (4) In Sweden it would be around 1 3 million (incidence of CF 1 3 000 (6) and of CD 1 1 000 (2)) The coexistence of these two diseases is thought to be chance (5) although CF might predispose to the development of CD (4) In a 10-year period (with 33 405 live births) we observed three children with exocrine pancreatic insufficiency and small intestinal dysfunction

Patient 1 CF and CD Boy only child of Jewish unrelated parent At age 1 month he had bronchopneumonia with atelectasis at 5 7 and 8 months bronchitis Gluten containing diet from age 4 months From 6 months diarrhoea failure to thrive and weight loss At 8 months two pilocarpine iontophoresis gave a sweat sodium of 1?3 and 106 mmol/l Duodenal juice had no trypsin and no amylase activities No *Giardia lamblia* Xylose and lactose tolerance tests abnormal Intestinal biopsy showed a morphology in agreement with coeliac disease intestinal disaccharidases (cf 3) very low (Table 1) He gained weight and dramatically improved on pancreatic extract and vitamins physiotherapy antibiotics and a gluten free diet At 13 months intestinal biopsy showed marked morphological improvement (Table 1) Intestinal dipeptidase activities (cf 3) against val glu and ala pro were extremely low (Table 1) Xylose test normal Gluten challenge at 15 months gave diarrhoea and decreased xylose absorption Two further gluten challenges at 4 and 5 years resulted in diarrhoea Now at age 6 5 years he has normal weight and height

Patient 2 CF and disaccharidase deficiency Girl second child of unrelated parents and with a healthy brother Recurrent respiratory infections from age 9 month diarrhoea and no weight gain Two pilocarpin iontophoresis at 1 months showed a sweat sodium of 100 and 9? mmol/l No trypsin activity detected in duodenal juice Xylose tolerance test normal lactose tolerance test abnormal Intestinal biopsy showed villous mucosa with inspissated mucous secretion in the crypts normal intestinal dipeptidases low disaccharidase activities (especially lactase activity) (Table 1) No symptoms of disaccharide intolerance Died at age 5 5 years

Patient 3 Shwachman syndrome food intolerance decreased intestinal lactase ala pro and val glu dipeptidase activities Boy only child of unrelated parents Admitted at age months for infantile colic and eczema He got diarrhoea and failed to thrive had neutropenia and slight thrombocytopenia On three occasions low or zero values of trypsin amylase and lipase in duodenal juice Pilocarpin iontophoresis ×3 gave a sweat sodium of 17 19 and 34 mmol/l Xylose and lactose tolerance tests normal at age 4 months Intestinal biopsy at 6 months showed a slightly damaged mucosa low val glu and ala pro dipeptidase activities and low lactase activity (Table 1) At 9 and 18 months urticaria after an egg and skin rash repeatedly after drinking milk At 3 years he tolerated egg at 6 years milk Height now at age 9 years 115 cm (=−3 S D)

The small intestinal dysfunction reported in CF includes besides CD specific lactase deficiency (1 8) reduced lactase activities (7 8) and reduced uptake of phenylalanine and cycloleucine (8) In Shwachman syndrome (10) the morphology of the intestinal mucosa was reported normal (7 10) or slightly changed (9 10) Intestinal disaccharidases (7 9) and enterokinase (7) normal

Besides eczema observed in Shwachman syndrome (9 10) our patient had allergic skin reactions after egg and milk also low intestinal lactase ala pro and val glu dipeptidase activities These two dipeptidase activities were also proportionally further decreased in the second biopsy from the boy with CF+CD

Abbreviations CF=cystic fibrosis CD=coeliac disease Gly leu=glycyl L leucine Ala glu=L alanyl L glutamic acid val glu=L valine L glutamic acid glu val=L glutamyl L valine ala pro=L alanyl L proline ala phe=L alanyl L phenylalanine

Grants from Swedish Medical Research Council (project no 5143 and 5364) supported this work

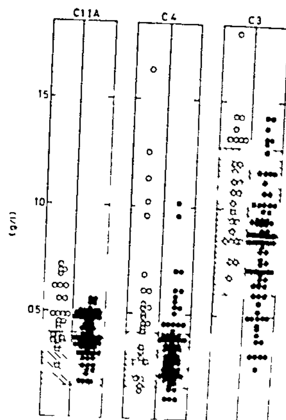


Fig 2 Serum concentrations of C1-esterase inactivator and complement factors C3 and C4 in children with acute lymphoblastic leukemia before treatment or during bone marrow relapse (O) and in remission either on or off treatment (●) Shaded area = reference ranges

figures for the complement factor C4 were for children with ALL before treatment or in relapse 0 54 g/l for children in remission either on or off treatment 0 33 g/l For C3 the results were children with ALL before treatment or in relapse 1 10 g/l and children in remission either on or off treatment 0 82 g/l The pre treatment or relapse values of C1 IA C3 and C4 were significantly different from those following successful therapy ($p<0.05$) The results from serial determinations of C1 IA in the five patients who relapsed are shown in Fig 1 Only 2 of the patients had elevated C1 IA at the time of relapse and in all of them there were large fluctuations of the C1 IA serum levels Similar observations were recorded for the other complement factors measured in the sera of these patients (data not shown) Subnormal serum lysozyme concentrations in patients with acute lymphoblastic leukemia have been regularly reported (4 5 7) Upon remission the lysozyme level usually returns to normal (7) According to Bratlid & Moe (4) serial determinations of lysozyme might be of value in leukemic children under treatment to assess the efficacy of therapy and to discover early systematic relapses Bach Mortensen et al (1 2) reported increasing C1 IA and C4 values in relapses of malignant diseases (including leukemia) and decreasing values during effective cytostatic treatment

The results here presented which are analogous to the fluctuations of serum ferritin in children with ALL (6) indicate that determinations of these serum components are of little assistance to detect or rule out relapse of ALL at an early stage

ACKNOWLEDGEMENT

The technical assistance of Mrs N Gade Christen and Mrs Eli M Jensen is greatly acknowledged T work was in part supported by a grant from the Norwe Cancer Society

M E Koller[1] B Haneberg R M
P H Finne I Romslo[1]

REFERENCES

1 Astrup J Colstrup H & Frandsen B Compleme C1 inactivator in the serum of patients with malignai disease Acta Radiol Therapy Physics Biol 16 39 1977
2 Bach Mortensen N Osther K & Strøyer I C1 esterase inactivator and C4 in malignant disease Lancet II 499 1975
3 Borgen J & Romslo I Lysozyme determined in serum and urine by a simple nephelometric method Clin Chemistry 23 1599 1977
4 Bratlid D & Moe P J Serum lysozyme activity in children with acute leukemia Eur J Pediatr 127 63 1978
5 Finch S C Castro O Lippman M E Donadio J A & Perillie P E Lysozyme in leukopathic states In Osserman E F Canfield R E & Beych k S (eds) Lysozyme Academic Press New York 19 4 p 335
6 Koller M E Romslo I Finne P H & Haneberg B Serial determination of serum ferritin in children with acute lymphoblastic leukemia Evaluation of it usefulness as a prognostic index Acta Paediatr Scand 68 93 1979
7 Moe P J Haneberg B & Finne P H Serum lysozyme activity in children with hematological and malignant disorders Acta Paediatr Scand 64 530 1975

Laboratory of Clinical Biochemistry [2]Department of Pediatrics and [3]Broegelmann Research Laboratory for Microbiology University of Bergen N 5016 Haukeland Sykehus Norway

Acta Pædiatr Scand 68 277-280 1979

CASE REPORT

HYPERBILIRUBINAEMIA AND IDIOPATHIC HYPOPITUITARISM IN THE NEWBORN PERIOD

S L S DROP E COLLE and H J GUYDA

From the Department of Paediatrics and Montreal Children's Hospital Research Institute Montreal Canada

ABSTRACT Drop S L S Colle E and Guyda H J (Department of Paediatrics and Montreal Children's Hospital Research Institute Montreal Canada) Hyperbilirubinaemia and idiopathic hypopituitarism in the newborn period Acta Paediatr Scand 68 277 1979 —Two infants with idiopathic panhypopituitarism presented with severe neonatal hypoglycaemia hepatomegaly and hyperbilirubinaemia (direct and indirect) Abnormal liver function tests returned to normal over a 5–8 month period The growth rate in the absence of detectable growth hormone was 50% of normal during the first 6 months The effect of growth hormone on somatomedin levels and growth rate during the first year of life in one of the infants is described

KEY WORDS Hyperbilirubinaemia growth hormone somatomedin hypopituitarism

Congenital hypopituitarism due to a deficiency of hypothalamic hypophysiotropic hormones or aplasia of the pituitary gland is a well recognized syndrome consisting of severe and persistent hypoglycaemia and microphallus in neonates of normal birth weight and length (2 11 14 16 18) In addition liver dysfunction with clinical and histologic evidence of the neonatal hepatitis syndrome has been described in some of these infants (12) To underscore the importance of early detection and treatment of this syndrome we present two patients in whom the diagnosis of hypopituitarism was made within the first months of life The linear growth rate in the absence of detectable growth hormone was 50% of normal during the first 6 months The effect of GH treatment on somatomedin levels and growth rate during the first year of life in one of the infants is described

CASE HISTORIES

Case 1

Patient J M was born after an uncomplicated term pregnancy with normal labor and delivery The Apgar score was 8 at 1 min and 10 at 5 min The birth weight was 3 76 kg length 50 cm head circumference 36 cm At 12 hours of age the baby had a generalized seizure A serum glucose was 1 27 mmol/l calcium 2 5 mmol/l Six hours later another seizure occurred with a glucose level of 0 33 mmol/l Physical examination disclosed an irritable oriental baby with reddish blond hair There was hepatomegaly (1 5 cm below the right costal margin) without splenomegaly Glucose levels remained labile requiring glucose infusions of 8 mg/kg body weight/minute until the 15th day of life Gradually normoglycaemia could be maintained with 3 hourly oral feedings

On the second day of life the infant became jaundiced Over the following 10 days she required 4 blood exchanges The highest recorded indirect serum bilirubin level was 340 μmol/l highest direct 78 6 μmol/l The blood type of mother and baby was A positive Peripheral blood smear from the parents and baby were normal Direct and indirect Coombs tests were negative Prior to the second exchange on the 4th day the serum LDH was 654 IU SGOT 87 IU alkaline phosphatase 5 IU These liver function tests remained unchanged throughout the hospital stay At three months of age the direct bilirubin was 59 85 μmol/l total 80 37 μmol/l SGOT 196 IU

She received a 10 day course of antibiotics despite negative bacterial cultures of blood urine and CSF An IgM level prior to the first exchange was 0 76 g/l Viral studies for rubella CMV RSV parainfluenza 1 2 3 as well as studies for toxoplasma in infant and mother were negative Fundoscopic examination of the eyes was normal The hepatitis B antigen was negative The sweat chloride serum alpha 1 antitrypsin alpha fetoprotein and G-6-PD levels were all normal The urine was negative for ketones and reducing substances Serum and urine amino acid chromatograms were normal A roentgenogram of the

Table 1 *Morphology intestinal dipeptidases and disaccharidases in small intestinal biopsies (duodeno jejunal flexure) from the three patients*

	1 CF+CD			
	On gluten	Without gluten	2 CF	3 Shwachman syndrome
Morphology				
Dissecting microscopy	Mosaic convoluted	Leaves+ridges	Finger+leaves	Leaves
Histology	Cuboidal and irregular epithelial cells Elongation of crypts Infiltration of plasma cells and leucocytes	Normal epithelial cells Elongation of crypts inspissated mucous secretion Infiltration of plasma cells	Normal epithelial cells Slight elongation of crypts inspissated mucous secretion Slight infiltration of plasma cells and leucocytes	Normal epithelial cells Slight elongation of crypts slight infiltration of plasma cells and leucocytes
Intestinal dipeptidases (u/mg N)				
Gly leu (98–350)[a]	–	71	223	119 226[b]
Ala glu (20–60)	–	17	60	21 –
Val glu (7 2–27)	–	<0 5	36	4 8 6 4
Glu val (7 2–41)	–	2 7	17	8 2 8 7
Ala pro (5 1–19)	–	0 8	13	1 0 1 9
Intestinal disaccharidases (u/g protein)				
Maltase (91–600)	16	–	24	160
Isomaltase (20–126)	7 8	–	9 5	54 4
Sucrase (23–166)	4 8	–	10 5	44 4
Trehalase (8 3–50)	0 5	–	2 2	5 3
Lactase (6 6–73)	0 8	–	1 1	5 8

[a] Controls range (n=49) (3) [b] Two biopsies from the same biopsy occasion

To conclude we found morphological and enzymatical abnormalities in intestinal mucosa in both CF and Shwachman syndrome favouring the hypothesis that exocrine pancreatic insufficiency predisposes to the development of small intestinal dysfunction in phenotypically high risk individuals

N O Berg *A Dahlqvist* *Tor Lindberg*

Departments of Pathology and Nutrition University of Lund and Departments of Paediatrics and Experimental Research Malmo General Hospital University of Lund Sweden

REFERENCES

1 Antonowicz I Reddy V Khaw K T & Shwachman H Lactase deficiency in patients with cystic fibrosis *Pediatrics 42* 492 1968
2 Berg N O & Lindberg T Incidence of coeliac disease and of transient gluten intolerance in a Swedish urban community *Acta Paediatr Scand 68* 1979 In press
3 Dahlqvist A Lindberg T Meeuwisse G & Åkerman M Intestinal dipeptidases and disaccharidases in children with malabsorption *Acta Paediatr Scand 59* 621 1970
4 Goodchild M C Nelson R & Anderson Ch M Cystic fibrosis and coeliac disease coexistence in two children *Arch Dis Child 48* 684 1973
5 Katz A J Falchuk M Z & Shwachman H The coexistence of cystic fibrosis and coeliac disease *Pediatrics 57* 715 1976
6 Kollberg H Cystic fibrosis in Sweden Thesis *Acta Universitatis Upsaliensis 19?* 1974
7 Lebenthal E Antonowicz I & Shwachman H Enterokinase and trypsin activities in pancreatic insufficiency and diseases of the small intestine *Gastroenterology 70* 508 1976
8 Morin C L Roy C C Lasalle R & Bonin A Small bowel dysfunction in patients with cystic fibrosis *J Pediat 88* 213 1976
9 Shmerling D H Prader A Hitzig W H Giedion A Hadorn B & Kuhni M The syndrome of exocrine pancreatic insufficiency neutropenia metaphyseal dysostosis and dwarfism *Helv Paediat Acta 24* 547 1969
10 Shwachman H Diamond L K Oski F A & Khaw K T The syndrome of pancreatic insufficiency and bone marrow dysfunction *J Pediat 65* 645 1964

Acta Pædiatr Scand 68 277-280 1979

CASE REPORT

HYPERBILIRUBINAEMIA AND IDIOPATHIC HYPOPITUITARISM IN THE NEWBORN PERIOD

S L S DROP E COLLE and H J GUYDA

Fr m the Department of Paedi trics and Montreal Childre s H spital Resea ch Institute M ntreal Canada

ABSTRACT Drop S L S Colle E and Guyda H J (Department of Paediatrics and Montreal Children s Hospital Research Institute Montreal Canada) Hyperbilirubinaemia and idiopathic hypopituitarism in the newborn period Acta Paediatr Scand 68 277 1979 —Two infants with idiopathic panhypopituitarism presented with severe neonatal hypoglycaemia hepatomegaly and hyperbilirubinaemia (direct and indirect) Abnormal liver function tests returned to normal over a 5–8 month period The growth rate in the absence of detectable growth hormone was 50% of normal during the first 6 months The effect of growth hormone on somatomedin levels and growth rate during the first year of life in one of the infants is described

KEY WORDS Hyperbilirubinaemia growth hormone somatomedin hypopituitarism

Congenital hypopituitarism due to a deficiency of hypothalamic hypophysiotropic hormones or aplasia of the pituitary gland is a well recognized syndrome consisting of severe and persistent hypoglycaemia and microphallus in neonates of normal birth weight and length (2 11 14 16 18) In addition liver dysfunction with clinical and histologic evidence of the neonatal hepatitis syndrome has been described in some of these infants (12) To underscore the importance of early detection and treatment of this syndrome we present two patients in whom the diagnosis of hypopituitarism was made within the first months of life The linear growth rate in the absence of detectable growth hormone was 50% of normal during the first 6 months The effect of GH treatment on somatomedin levels and growth rate during the first year of life in one of the infants is described

CASE HISTORIES

Ca 1

Patient J M was born after an uncomplicated term pregnancy with normal labor and delivery The Apgar score was 8 at 1 min and 10 at 5 min The birth weight was 3 6 kg length 50 cm head circumference 36 cm At 12 hours of age the baby had a generalized seizure A serum glucose was 1 .7 mmol/l calcium 25 mmol/l Six hours later another seizure occurred with a glucose level of 0 33 mmol/l Physical examination disclo ed an irritable oriental baby with reddish blond hair There was hepatomegaly (1 5 cm below the right costal margin) without splenomegaly Glucose levels remained labile requiring glucose infusions of 8 mg/kg body weight/minute until the 15th day of life Gradually normoglycaemia could be maintained with hourly oral feedings

On the second day of life the infant became jaundiced Over the following 10 days she required 4 blood exchanges The highest recorded indirect serum bilirubin level was 340 μmol/l highest direct 78 6 μmol/l The blood type of mother and baby was A positive Peripheral blood smear from the parents and baby were normal Direct and indirect Coombs tests were negative Prior to the second exchange on the 4th day the serum LDH was 654 IU SGOT 87 IU alkaline phosphatase 5 IU These liver function tests remained unchanged throughout the hospital stay At three months of age the direct bilirubin was 59 85 μmol/l total 80 37 μmol/l SGOT 196 IU

She received a 10 day course of antibiotics despite negative bacterial cultures of blood urine and CSF An IgM level prior to the first exchange was 0 76 g/l Viral studies for rubella CMV RSV parainfluenza 1 2 3 as well as studies for toxoplasma in infant and mother were negative Fundoscopic examination of the eyes was normal The hepatitis B antigen was negative The sweat chloride serum alpha 1 antitrypsin alpha fetoprotein and G-6-PD levels were all normal The urine was negative for ketones and reducing substances Serum and urine amino acid chromatograms were normal A roentgenogram of the

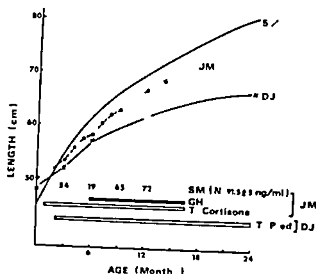


Fig 1 Growth data in J M and D J is plotted using the 5th percentile from the National Center for Health Statistics Growth Chart as reference The values for somatomedin radioreceptor activity (21) measured in plasma of J M are indicated by the numbers of the appropriate sampling times Replacement therapy is indicated by the bars

skull revealed no abnormalities The bone age at 1 week of age was consistent with term maturity

On the 25th day of life a needle biopsy of the liver was performed It showed a few intrasinusoidal foci of extramedullary haematopoiesis Portal triads and peri portal areas showed sparse infiltrate and round inflammatory cells occasionally arranged around bile canaliculi Hyperplasia of Kupffer cells was also present

On the 28th day of life cortisone acetate 5 mg t i d was started followed 2 days later by l thyroxine 25 µg/d with an increment to 37 5 µg/d at two months of age At 6 months of age GH treatment was initiated in a dose of 1 U i m 3 times per week (provided by the Medical Research Council of Canada) The effects on growth rate and somatomedin levels are depicted in Fig 1

Case 2

Patient D J a Caucasian boy was born of a 24 year old G II P O mother At 12 weeks gestation the mother had a flu like illness with vaginal spotting Two weeks prior to delivery the mother had another flu like illness with a diffuse maculo papular rash over her body and extremities including palms and soles as well as lymphadenopathy and joint pains Ampicillin was given Because of low urinary estriol levels (4 and 4 7 mg/24 hr) delivery was per Caesarian section at 43 weeks gestation Apgar score was 4 at 1 min 7 at 6 min The birth weight was 3315 g length 48 cm head circumference 37 cm On physical examination a full fontanelle measuring 4×2 cm with separation of the saggital sutures was noted Liver and spleen were both palpable 2 cm below the costal margin The testes were descended the phallus was very small At 2 hours of age a serum glucose level was 0 77 mmol/l Recurrent hypoglycaemia was treated with intravenous glucose infusions up to 6 mg/kg body weight/minute for the first 2 weeks of life Two hourly oral feedings co[uld] be introduced after 1 week

On the 2nd day of life the patient developed a macul[o] papular rash lasting 10 days Antibiotics were given H[e] was also noted to be jaundiced and required an exchan[ge] transfusion on day 2 (maximum serum bilirubin levels ind[i]rect 311 22 µmol/l direct 47 9 µmol/l) Indirect values de[creased] very gradually over the next two months whi[le] direct values climbed initially over the first ? weeks t[o] 133 4 µmol/l before returning to normal by 8 months o[f] age Liver enzymes prior to the exchange were SGO[T] 176 IU SGPT 112 IU and LDH 1580 IU and highest level[s] were recorded at 2 months of age (SGOT 670 IU SGP[T] 357 IU LDH 1725 IU) Normal values were obtained a[t] 8 months of age

The blood type of mother and baby was A positive Direct and indirect Coombs tests were negative The peripheral blood smear was normal Bacterial and viral cultures of throat urine and stool remained negative A serum IgM level (prior to exchange) was 0 08 g/l Fundoscopic examination of the eyes was normal Serology for mumps rubella CMC herpes varicella and toxoplasmosis in mother and baby remained negative Serum alpha 1 antitrypsin and a G-6-PD level were normal The hepatitis B antigen was negative The urine was negative for ketones and reducing substances A serum and urine amino acid chromatogram was normal

At 8 weeks of age an open liver biopsy was performed It showed moderately marked giant cell transformation with numerous bile thrombi in Zenker fixed material compatible with the diagnosis of giant cell hepatitis A roentgerogram of the skull showed a small flat pituitary fossa

At 2 months of age prednisone in an initial dose of 3 mg t i d and l thyroxine 50 µg/d were started After 1 month of treatment the prednisone dose was decreased to 1 mg t i d The patient remained prone to hypoglycaemia especially during intercurrent illnesses and prolonged fasting From 2½ years of age the patient received GH treatment according to the protocol of the Medical Research Council of Canada (9) Details of the endocrinologic data on both patients are given in Table 1

DISCUSSION

The relationship between hypopituitarism an[d] liver dysfunction with prolonged jaundic[e] noted in several previous reports and reviewe[d] by Herman (12) remains unclear It has bee[n] hypothesized that the absence of hormone(s) normally released by the pituitary might be re[s]ponsible Hypothyroidism secondary to TSH deficiency results in elevation of indirect bili rubin levels due to failure of conjugation in the absence of increased hemolysis Cortico steroids have been reported to increase the enterohepatic circulation of bile salts (17)

Table 1 *Endocrinologic evaluation*

Determinations were made in plasma or serum during the first weeks of life before hormonal replacement

		J M	D J
	Range of normal values		
T4	77–141 mM/l	54	46
TSH	<10×10 ³ int μ/l	6	
Thyroidal I uptake	8–30%		1% at 24 hrs
Idem following TSH	5 U thyropar® i m		12 9% at 6 hrs
	increase of 10–20%		17 4% at 24 hrs
Cortisol 8 a m	33 nM/d	8 3	
		12 4	
Cortisol following ACTH	6 5 U *cortrosyn® i m*		
	–3 fold increase	– fold	
Insulin glucose ratio	<50	14 2	
		10 7	
b Provocative tests	Peak values		
Glucagon (0 1 mg i m)	Glucose	3 6 mM/l	2 09 mM/l
	GH (>5 ng/ml)	1 ng/ml	1 ng/ml
Arginine (500 mg/kg i v over 30 min)	GH (>5 ng/ml)	1 ng/ml	1 ng/ml
TRH (100 μg i v)	GH (<5 ng/ml)	2 5 ng/ml	1 ng/ml
	PRL (>15 ng/ml)	– 8 ng/ml	4 0 ng/ml
	TSH (>10 μU/ml)	21×10 int μ/l	12×10 ³ int μ/l

Range of normal for first week of life

Performed in J M at 7 months of age in D J at 5½ years thyroid medication was *discontinued* 2 weeks *prior* to testing TSH T4 GH PRL and cortisol were determined by radioimmunoassay

However hyperbilirubinaemia and abnormal liver function tests persisted in these two infants after *replacement therapy* with cortisol and thyroxine was instituted a rise in the direct bilirubin characterized this period and an increase in liver *enzymes* Studies *in* rats suggested that growth hormone can modulate bile acid synthesis (1) A single study in humans deficient in growth hormone revealed *increased* micellar phase bile acid in the jejunum following *GH* treatment Whether the increase was *due to the increased* bile acid synthesis *or* secretion could not be determined from the design of the study Decreased bile acid secretion could lead to cholestasis It is of interest that bile acid concentrations normally increase with age and this could *explain the spontaneous* resolution of the abnormal liver function noted *in* these patients (17)

In both patients evidence of hypothyroidism and hypoadrenalism was present in the first weeks of *life* Although no growth hormone could be detected upon stimulation with glucagon and arginine a growth rate of approximately 50% of normal during the first 4–5 months was observed

The initiation of GH treatment in patient J M resulted in an increase of SM levels and a linear growth rate of up to 80% of normal in sharp contrast to the growth pattern of patient D J who did not receive GH treatment until age 2½ (Fig 1)

Somatomedin serum peptides with insulin like activities appear to play an important role in fetal and neonatal growth (4 5 7) Patients *born with primary SM deficiency* are retarded in length at birth (15 20) A normal initial growth pattern generally seen in GH deficient infants (11 12 16) suggests that significant longitudinal growth can occur in the absence of GH The finding of a SM level 50% of normal at 3 months of age *in one of our* patients is in full agreement with the hypothesis that not all SM production is dependent on stimulation by GH (4) There is experimental evidence that PRL and ovine placental lactogen *can stimulate* hepatic SM generation in the rat (6 13) and the human (10) As well insulin an

important fetal growth factor was shown to sustain SM release in the perfused rat liver as effectively as GH (4)

In contrast to the report of Lovinger (18) basal PRL levels were low and the response to TRH was subnormal However TSH release following TRH was normal in both patients suggesting hypothalamic dysfunction

The two cases presented illustrate the importance of early diagnosis which in the male infant is facilitated by the apparent microphallus Complete hormonal replacement therapy including GH is essential not only to relieve symptomatic hypoglycemia but also to prevent a permanent growth delay at an age at which the response to GH is most favourable (9)

ACKNOWLEDGEMENTS

We gratefully acknowledge Drs K Scott Grace Maternity Hospital and R Goldbloom the Izack Walton Killam Hospital for Children Halifax N S for providing us with clinical data of patient D J We wish to thank Mrs Jean Parado for technical assistance

REFERENCES

1 Beher W T Beher M E & Semenuk G The effect of pituitary and thyroid hormones on bile acid metabolsim in the rat *Metabolism 15* 181 1966
2 Caccian E Cicognani A Piracolli P Tassoni P Bernardi F Sabardi S Zappulla F & Mazzanti L Congenital hypopituitarism associated with neonatal hypoglycemia and microphallus Effect of GH therapy *Helv Paediatr Acta 31* 481 1976
3 Daughaday W H Hormonal regulation of growth by somatomedin and other tissue growth factors *Clin Endocrinol 6* 117 1977
4 Daughaday W H Phillips L S & Mueller M C The effect of insulin and growth hormone on the release of somatomedin by the isolated rat liver *J Lab Clin Med 7?* 803 1976
5 d Ercole A J Foushee D B & Underwood L E Somatomedin C receptor ontogeny and levels in porcine fetal and human cord serum *J Clin Endocrinol Metab 43* 1069 1976
6 Francis M J O & Hill D J Prolactin stimulated production of somatomedin by rat liver *Nature 255* 167 1975
7 Gluckman P D & Brinsmead M W Somatomedin in cord blood relationship to gestational age and birth size *J Clin Endocrinol Metab 43* 1378 1976
8 Guyda H J Heterogeneity of hGH and prolactin secreted in vitro Immunoassay and radioreceptor assay correlations *J Clin Endocrinol Metab 41* 953 1975
9 Guyda H J Friesen H Bailey J D Leboeuf G & Beck J C Medical Research Council of Canada Therapeutical trial of human growth hormone first 5 years of therapy *Can Med Assoc J 11?* 1301 1975
10 Guyda H J Corvol M T Rappaport R & Posner B I Radioreceptor assay of insulin like peptides in human plasma growth hormone dependence and correlation with sulphation activity Submitted for publication to *J Clin Endocrinol Metab*
11 Henze G Ruhl R & Werner E Kombinierte hypophysäre Insuffizienz beim Säugling *Mschr Kinderheilkd 124* 398 1976
12 Herman S P Baggenstoss A H & Cloutier M D Liver dysfunction and histologic abnormalities in neonatal hypopituitarism *J Pediatr 8?* 89? 197?
13 Hurley T W d Ercole A J Handwerger S Underwood L E Furlanetto R W & Fellows R E Ovine placental lactogen induces Somatomedin a possible role in fetal growth *Endocrinol ? 101* 1635 1977
14 Johnson J D Hansen R C Albritton W L Werthemann U & Cristiansen R O Hypoplasia of the anterior pituitary and neonatal hypoglycemia *J Pediatr 8? 634* 1973
15 Laron Z Karp M Pertzelan A Kauli R Keret R & Doron M The syndrome of familial dwarfism and high plasma immunoreactive human growth hormone *In* M Pecile & E E Muller (eds) *Growth and growth hormone* Excerpta Medica Amsterdam 1972 p 458
16 Lovinger R D Kaplan S L & Grumbach M M Congenital hypopituitarism associated with neonatal hypoglycemia and microphallus Four cases secondary to hypothalamic hormone deficiencies *J Pediatr 87* 1171 1975
17 Poley J R Smith J D Thompson J B & Seely J R Improved micellar dispersal of dietary lipid by bile acids during replacement therapy in growth hormone deficient children *Pediatr Res 1?* 1186 1977
18 Sadeghi Nejad A & Senior B A familial syndrome of isolated aplasia of the anterior pituitary *J Pediatr 84* 79 1974
19 Schiffrin A Guyda H Robitaille P & Posner B Increased plasma somatomedin reactivity in chronic renal failure as determined by acid gel filtration and radioreceptor assay *J Clin Endocrinol Metab 46* 511 1978
20 Van den Brande J L du Caju M V L Visser H K A Schopman W Hackeng W H L & Degenhart H J Primary somatomedin deficiency Case report *Arch Dis Child 49* 29? 1974

Submitted June 12 1978
Accepted Sept 8 1978

(E C) Department of Paediatrics
Montreal Children s Hospital
Research Institute
2300 Tupper Street
Montreal Quebec
Canada H3H 1P3

CASE REPORT

CONGENITAL ASCITES DUE TO MESENTERIC VESSEL CONSTRICTION CAUSED BY MALROTATION OF THE INTESTINES

J HERTEL and P VOLSTED PEDERSEN

From the Departments of Neonatology and Paediatric Surgery Rigshospitalet Copenhagen Denmark

ABSTRACT Hertel J & Volsted Pedersen P (Departments of Neonatology and Paediatric Surgery University Hospital Copenhagen Denmark) Congenital ascites due to mesenteric vessel constriction caused by malrotation of the intestines Acta Paediatr Scand 68 281 1979.—A newborn premature girl with congenital non-chylous ascites is presented The ascites recurred although laparocentesis was performed three times The ascites was probably due to a superior mesenteric vein constriction caused by a malrotation of the intestines After division of the Ladd bands no ascites recurred Non-chylous congenital ascites may have a surgically treatable cause

KEY WORDS Neonates non-chylous ascites malrotation of the intestines mesenteric vessel constriction portal hypertension

The relatively rare condition of congenital ascites has been described occurring in connection with obstructive uropathy meconium peritonitis hydrometrocolpos fetal cytomegalic inclusion disease chylous ascites pancreatitis polycystic kidneys or liver Wilm s tumor neuroblastoma organomegaly and anterior meningocele (4)

We present a case of congenital ascites which was possibly caused by constriction of the superior mesenteric vessels and disappeared after operation for malrotation of the intestines

CASE STORY

A female infant was delivered after 37 weeks of gestation The pregnancy had been complicated by bleeding in the first month and by mild pre-eclampsia which was treated with diuretics and sedatives during the last month of the pregnancy The mother was 34 years old and this was her first pregnancy

Five days before delivery an ultrasound scan disclosed fetal ascites and she was admitted to this hospital where labour started shortly after but was stopped by Utopar® Betamethasone was also given 5 days later labour recommenced but this time could not be stopped Delivery was uncomplicated and the Apgar scores were 7 after 1 min and 9 after 5 min Birthweight was 1910 g and the length 41 cm

The infant had a large abdomen with a cone shaped thorax and very thin extremities with a mild equinovarus malformation of both feet The external genitalia were normal Voiding was spontaneous and sterile at culture Because of respiratory distress a laparocentesis was performed and 750 ml of clear yellow sterile fluid was obtained The abdominal wall was now very lax and no intra abdominal abnormalities could be palpated

Oral feeding with Pregestimil® (a formula containing medium chain triglycerides glucose and hydrolysed protein) was instituted together with intravenous glucose-saline over the next few days her oral intake varied During this time she passed loose stools daily without blood and never showed any signs of ileus At 10 days of age she could only take very little orally and the weight had decreased to 1490 g Parenteral nutrition was started

Although diuretics and albumin were given the abdominal circumference increased and laparocentesis was performed when she was 3 and 4 weeks old yielding 80 and 180 ml of clear fluid respectively The ascites contained no fat very few cells and the concentrations of sodium and potassium were equal to those of serum In the first laparocentesis the protein concentration was 2 95 g/l In the second laparocentesis the albumin concentration was 278 nmol/l while the serum concentration was 315 nmol/l A test for amylase was not performed on the ascitic fluid An intravenous pyelogram was judged as normal as were the ureters and the bladder A barium

follow through of the gastrointestinal tract disclosed a malrotation of the intestines without any signs of stenosis An angiocardiography showed the inferior caval vein to be normal and an aortography disclosed a slightly enlarged spleen and a normal liver

At 1 month of age an exploratory laparotomy was performed because of recurrent ascites continuous loss of weight and failure to respond to medical treatment

The intestines were found dilated and edematous and they were covered with fibrinous adhesions The sparse ascitic fluid was sterile at culture The intestinal tract was malrotated with the third part of the duodenum in front of the superior mesenteric vessels which were slightly compressed by the duodenum It was noted that a slight traction on the upper part of the small intestine caused ischaemia of the most distal part of the duodenum and the upper part of the jejunum The pancreas the liver the kidneys the bladder and the internal genitals all looked normal but the spleen was slightly enlarged The Ladd bands were divided and after that no vascular insufficiency was observed

After the operation intravenous nutrition was continued and oral feeding was restarted after 3 days 2 weeks later she was tolerating oral feeding and was gaining weight She was discharged from the neonatal department when 3 months of age weighing 2480 g At follow up she continued to thrive and no signs of ascites were seen At 18 months of age an intravenous pyelogram and a cystourography was performed because of pyuria and disclosed bilateral vesico ureteral reflux and a slight dilatation of the right ureter

DISCUSSION

In an experimental study on dogs Witte et al (9) in 1969 showed that ascites developed after extrahepatic portal hypertension had been produced by a shunt between the aorta and the portal vein combined with restriction to the trans hepatic blood flow Intestinal edema also developed The protein content of the ascites was low and almost exclusively albumin This was in contrast to experimentally induced chronic constriction of the inferior caval vein where the ascites produced was of high protein content and contained a large globulin fraction

Chylous ascites is characterized by milky ascites especially after meals containing fat The etiology is unknown in most cases but it is often a self limiting disease The treatment consists of supportive measures with intravenous nutrition and an oral diet containing short chain triglycerides and high protein (7 8) The long term prognosis is usually good though Sanchez et al (7) noted central ner vous system aberrations in 3 of 11 infants wit chylous ascites

Non chylous congenital ascites with lowe urinary tract obstruction has been known for long time (5) About 50% of all cases of co genital ascites have accompanying urinar tract abnormalities (6) The ascites may be du to leakage or transudation of urine from th bladder the ureters or the kidneys The pr tein content in the ascites is usually lo (3 6)

A few cases of ascites due to pancreat fluid leakage into the peritoneal cavity hav been reported (2) Pancreatitis is an unusu condition in infancy most often caused by at dominal trauma Other possible causes a hereditary pancreatitis ascaris infestatio virus infection steroid medication (1) hype lipidemia and abnormalities of the pancreat ducts (4) In our patient the pancreas wa found to be normal at operation There wer no signs of major genito urinary abnormaliti in the neonatal period Urine was passed no mally and was normal except for a slight pr teinuria She later developed bilateral reflu and dilatation of the right ureter

The ascites in our patient was never ch lous The first laparocentesis was clear bu was performed before any food had bee given The next two laparocenteses were pe formed after breast milk had been given an the ascites would thus have been milky if had been of the chylous type

The observations during operation of supe rior mesenteric vessel constriction and c ischaemia caused by traction on the Ladd bands and on the intestines are consistent wit ascites due to extrahepatic portal hypertensio combined with restriction of the transhepat blood flow The protein content of the flui and the normal appearance of the liver alon with slight enlargement of the spleen suppor this hypothesis After the constriction was re leased no ascites was seen

Non chylous congenital ascites may have a surgically treatable cause and an exploratory

laparotomy should be performed if medical supportive treatment and repeated laparocenteses are not successful

REFERENCES

1 Baar H S & Wolff O H Pancreatic necrosis in cortisone treated children *Lancet I* 81 1957
Coupland G Pancreatic ascites A rare complication of pancreatitis in childhood *Aust NZ J Surg 40* 5 1971
3 France N E & Back E H Neonatal ascites associated with urethral obstruction *Arch Dis Child 29* 565 1954
4 Gryboski J *Gastrointestinal Problems in the Infant* p 183 Saunders Philadelphia 1975
5 Marx K & Dale W A Neonatal ascites and obstructive uropathy *Pediatrics* 27 9 1961
6 Radman H M Dystocia due to fetal abdominal enlargement *Obstet Gynecol 19* 481 196
7 Sanchez R E Mahour G H Brennan L P & Wooley M M Chylous ascites in children *Surgery 69* 183 1971
8 Sturmer jr F C Infantile chylous ascites *Am Surg 31* 81 1965
9 Witte C L Chung Y C Witte M H Sterle O F & Cole W R Observations on the origin of ascites from experimental extrahepatic portal congestion *Ann Surg 170* 1007 1969

Submitted Aug 21 1978
Accepted Sept 26 1978

(J H) Department of Paediatrics G 4062
Rigshospitalet
DK 2100 Copenhagen Ø
Denmark

follow through of the gastrointestinal tract disclosed a malrotation of the intestines without any signs of stenosis An angiocardiography showed the inferior caval vein to be normal and an aortography disclosed a slightly enlarged spleen and a normal liver

At 1 month of age an exploratory laparotomy was performed because of recurrent ascites continuous loss of weight and failure to respond to medical treatment

The intestines were found dilated and edematous and they were covered with fibrinous adhesions The sparse ascitic fluid was sterile at culture The intestinal tract was malrotated with the third part of the duodenum in front of the superior mesenteric vessels which were slightly compressed by the duodenum It was noted that a slight traction on the upper part of the small intestine caused ischaemia of the most distal part of the duodenum and the upper part of the jejunum The pancreas the liver the kidneys the bladder and the internal genitals all looked normal but the spleen was slightly enlarged The Ladd bands were divided and after that no vascular insufficiency was observed

After the operation intravenous nutrition was continued and oral feeding was restarted after 3 days 2 weeks later she was tolerating oral feeding and was gaining weight She was discharged from the neonatal department when 3 months of age weighing 2480 g At follow up she continued to thrive and no signs of ascites were seen At 18 months of age an intravenous pyelogram and a cystourography was performed because of pyuria and disclosed bilateral vesico ureteral reflux and a slight dilatation of the right ureter

DISCUSSION

In an experimental study on dogs Witte et al (9) in 1969 showed that ascites developed after extrahepatic portal hypertension had been produced by a shunt between the aorta and the portal vein combined with restriction to the trans hepatic blood flow Intestinal edema also developed The protein content of the ascites was low and almost exclusively albumin This was in contrast to experimentally induced chronic constriction of the inferior caval vein where the ascites produced was of high protein content and contained a large globulin fraction

Chylous ascites is characterized by milky ascites especially after meals containing fat The etiology is unknown in most cases but it is often a self limiting disease The treatment consists of supportive measures with intravenous nutrition and an oral diet containing short chain triglycerides and high protein (7 8) The long term prognosis is usually good though Sanchez et al (7) noted central nervous system aberrations in 3 of 11 infants with chylous ascites

Non chylous congenital ascites with lower urinary tract obstruction has been known for a long time (5) About 50% of all cases of congenital ascites have accompanying urinary tract abnormalities (6) The ascites may be due to leakage or transudation of urine from the bladder the ureters or the kidneys The protein content in the ascites is usually low (3 6)

A few cases of ascites due to pancreatic fluid leakage into the peritoneal cavity have been reported (2) Pancreatitis is an unusual condition in infancy most often caused by abdominal trauma Other possible causes are hereditary pancreatitis ascaris infestation virus infection steroid medication (1) hyperlipidemia and abnormalities of the pancreatic ducts (4) In our patient the pancreas was found to be normal at operation There were no signs of major genito urinary abnormalities in the neonatal period Urine was passed normally and was normal except for a slight proteinuria She later developed bilateral reflux and dilatation of the right ureter

The ascites in our patient was never chylous The first laparocentesis was clear but was performed before any food had been given The next two laparocenteses were performed after breast milk had been given and the ascites would thus have been milky if it had been of the chylous type

The observations during operation of superior mesenteric vessel constriction and of ischaemia caused by traction on the Ladd bands and on the intestines are consistent with ascites due to extrahepatic portal hypertension combined with restriction of the transhepatic blood flow The protein content of the fluid and the normal appearance of the liver along with slight enlargement of the spleen support this hypothesis After the constriction was released no ascites was seen

Non chylous congenital ascites may have a surgically treatable cause and an exploratory

laparotomy should be performed if medical supportive treatment and repeated laparocenteses are not successful

REFERENCES

Baar H S & Wolff O H Pancreatic necrosis in cortisone treated children *Lancet 1* 812 1957

Coupland G Pancreatic ascites A rare complication of pancreatitis in childhood *Aust NZ J Surg 40* 25 1971

3 France N E & Back E H Neonatal ascites associated with urethral obstruction *Arch Dis Child* 29 565 1954

4 Gryboski J *Gastrointestinal Problems in the Infant* p 183 Saunders Philadelphia 1975

5 Marx K & Dale W A Neonatal ascites and obstructive uropathy *Pediatrics 27* 9 1961

6 Radman H M Dystocia due to fetal abdominal enlargement *Obstet Gynecol 19* 481 1962

7 Sanchez R E Mahour G H Brennan L P & Wooley M M Chylous ascites in children *Surgery 69* 183 1971

8 Sturmer jr F C Infantile chylous ascites *Am Surg 31* 281 1965

9 Witte C L Chung Y C Witte M H Sterle O F & Cole W R Observations on the origin of ascites from experimental extrahepatic portal congestion *Ann Surg 170* 100 1969

Submitted Aug 21 1978
Accepted Sept 26 1978

(J H) Department of Paediatrics G 406
Rigshospitalet
DK 2100 Copenhagen Ø
Denmark

Acta Pædiatr Scand 68 285–286 1979

CASE REPORT

HYPOGLYCAEMIA AND CONGENITAL ADRENAL HYPERPLASIA

M GEMELLI F DE LUCA and G BARBERIO

From the 2nd Department of Paediatrics University Hospital Messina Italy

ABSTRACT Gemelli M De Luca F and Barberio G (2nd Department of Paediatrics University Hospital Messina Italy) Hypoglycaemia and congenital adrenal hyperplasia Acta Paediatr Scand 68 285 1979 —We report the case history of a child with congenital adrenal hyperplasia which was complicated by recurrent hypoglycaemic episodes during common infections There are few reports in literature on the association of hypoglycaemia and congenital adrenal hyperplasia In accordance with others we believe that hypoglycaemic attacks in adrenal hyperplasia are perhaps more frequent than recorded in literature

KEY WORDS Hypoglycaemia adrenal hyperplasia

As hypoglycaemia is not a well recognized complication of 21 hydroxylase deficiency we consider it useful to report the case history of a child with a salt losing congenital adrenal hyperplasia and frequent hypoglycaemic attacks

CASE REPORT

D A was born in Messina to parents who are first cousins The mother bore four sons The first two died during the first month of life because of uncontrollable vomiting and diarrhoea the third is well and the fourth was admitted to our Clinic in the first hours of life with respiratory distress During the hospitalization he had seizure vomiting dehydration and skin pigmentation The clinical and laboratory data (plasma Na 94 mmol/l K 7 7 mmol/l cortisol 30 μg/l 17 hydroxyprogesterone 85 μg/l urinary 17 ketosteroids 9 1 mg/ 4 h) suggested the diagnosis of salt losing congenital adrenal hyperplasia The symptoms rapidly disappeared and the biochemical parameters returned to normal after treatment with 9 α fluorohydrocortisone (0 1 mg/day) and cortisone acetate (40 mg/m /day) was begun

Since then his growth has been good and the plasma electrolytes and 17 hydroxyprogesterone urinary 17 ketosteroid and bone age have always been normal

When he was aged 7 months during an attack of gastroenteritis the patient showed changes of mood drowsiness perspiration and had generalized seizures He was immediately hospitalized and underwent laboratory investigations The plasma electrolytes were normal but the blood glucose was 1 11 mmol/l The hypoglycaemia and convulsions were rapidly controlled by intravenous glucose During this hospitalisation an intravenous insulin tolerance test was performed (4 U/m) This provoked marked hypoglycaemia (1 16 mmol/l) with pallor and sweating but the glucose recovery index was only slightly decreased (170%) This test revealed normal plasma growth hormone (maximum 15 ng/l) and glucagon (maximum 90 ng/l) responses and a normal increase of urinary epinephrine (680%) in the three hours after the insulin injection while plasma cortisol remained substantially unchanged (from 38 to 41 μg/l)

Since then following our suggestions the child s parents have given him hydrocortisone by injection and sweetened drinks whenever they have observed any change of mood drowsiness or increased sweating which have generally occurred during intercurrent infections

In spite of these precautions during the last three years the child has six times developed more or less serious neurological symptoms such as drowsiness convulsions and coma in association with common infections

On these occasions marked hypoglycaemia (0 55–1 38 mmol/l) was present while the electrolytes were always normal Glucose infusion provoked rapid resolution of the symptoms The seizures persisted several hours on only one occasion when the child was taken to another hospital The doctor on duty probably not knowing the risk of hypoglycaemia merely injected him with phenobarbital which was ineffective in controlling the convulsions It is notable that all the electroencephalograms to date have always been normal

DISCUSSION

Hypoglycaemia has been very rarely reported in association with 21 hydroxylase deficiency (4 6–9) which is not generally listed among the common causes of hypoglycaemia (1 5) Hypoglycaemic attacks occurred in only 2 out of 140 cases of adrenal hyperplasia followed up by Wilkins (8) According to Sauls & Ulstrom (6) congenital adrenal hyperplasia accounts for 4% of cases of spontaneous hypoglycaemia Job et al (2) reviewing the acute symptoms occurring over 6 years in 11 children with congenital 21 hydroxylase deficiency report no hypoglycaemic episodes In a review by Zuppinger (9) the occurrence of hypoglycaemia in children with adrenal hyperplasia is 1 40 Limbeck & Kelley (3) confirm the extremely infrequent reports of this complication which they expected to be more frequent considering the importance of cortisol in the maintenance of blood glucose Mackinnon & Grant (4) in 1977 reported two cases of 21 hydroxylase deficiency with hypoglycaemic seizures and emphasized that the risk of hypoglycaemia in this condition needs more accurate evaluation They suggested that this complication is perhaps more frequent than recorded in literature since it may at times be misinterpreted Their hypothesis seems to be confirmed by the history of our patient in whom hypoglycaemic convulsions occurred fairly frequently as a consequence of the stress of infection and in whom they were once wrongly attributed to epilepsy

REFERENCES

1 Ichiba Y & Bargman G J Hypoglycemia In J Gardner (ed) *Endocrine and genetic disease of childhood and adolescence* W B Saunders & Philadelphia 1975 p 931

2 Job J C Canlorbe P Lambertz J Couroubl & Rossier A Les accidents aigus au cours traitement de l hyperplasie surrenale congenitale sante par défaut de 21 hydroxilase *Ann Pediatr* 476 1970

3 Limbeck G & Kelley V Congenital adrenal hyperplasia *In* V C Kelley (ed) *Metabolic endocrine genetic disorders of children* Hagerstown (Maryl 1974 p 765

4 Mackinnon J & Grant D B Hypoglycaemia in congenital adrenal hyperplasia *Arch Dis Child* 52 1977

5 Pagliara A S Karl I E Haymond M & Ki D M Hypoglycemia in infancy and childhood *Pediatr* 82 365 1973

6 Sauls H S Jr & Ulstrom R A Hypoglyce *In* V C Kelley (ed) *Brenneman s Practice of pe trics* W F Prior Co Hagerstown Maryland vol 1

7 White F P & Sutton L E Adrenogenital syndr with associated episodes of hypoglycemia *J Clin docrinol 11* 1395 1951

8 Wilkins L *The diagnosis and treatment of endoc disorders in childhood and adolescence* Thor Springfield Ill 1965 3rd ed p 546

9 Zuppinger K A Hypoglycemia caused by dimini gluconeogenesis *In* K A Zuppinger (ed) *Hypogl mia in childhood Evaluation of diagnostic procedu* S Karger A G Basel Switzerland 1975 p 10

Submitted May 17 1978
Accepted Sept 17 1978

(F D L) * Clinica Pediatrica
Policlinico Universitario
98100 Messina
Italia

Acta Pædiatr Scand 68 287–289 1979

CASE REPORT

STRESS POLYCYTHAEMIA AND PERIPHERAL FACIAL PALSY COMPLICATIONS OF SEVERE HYPERTENSION

P M ZEIS S RAO E G JOHN and L C ASCHINBERG

F n the Department of Paediatrics Unn ersity of Illinois Hospital Chicag USA

ABSTRACT Zeis P M Rao S John E G and Aschinberg L C (Department of Paediatrics University of Illinois Hospital Chicago USA) Stress polycythaemia and peripheral facial palsy complications of severe hypertension Acta Paediatr Scand 68 287 1979.—An 11 month old boy had an episode of generalized convulsions followed by a right peripheral facial palsy which resolved gradually within 3 weeks Three months later he had another similar episode of convulsions followed by a left peripheral facial palsy On both occasions it was found that he had polycythaemia A careful physical examination discovered that the child had severe hypertension Extensive laboratory investigations did not reveal a cause for his hypertension Haematologic investigations showed that the polycythaemia was due to a contracted plasma volume as a result of the hypertension The peripheral facial palsy most probably was due to a blood clot in the facial canal below the origin of the nerve to m stapedius as audiograms were normal and lacrimation preserved Control of the hypertension resulted in resolution of the facial palsy within 4 weeks and normal haematocrit readings within 6 weeks It should be stressed that every patient with peripheral facial palsy should be examined for hypertension

KEY WORDS Hypertension polycythaemia facial palsy

Polycythaemia has been reported in adults as an infrequent complication of hypertension (2) Another complication of severe hypertension more common in adults than in the paediatric age group is peripheral facial palsy (4 5)

We report a 14 month old boy with polycythaemia and peripheral facial palsy who was found to have severe hypertension

CASE REPORT

A 14 month-old boy was admitted to the University of Illinois Hospital with the diagnosis of malignant hypertension The boy was well until the age of 11 months when he had an episode of generalized convulsions followed by a right peripheral facial palsy for which he was admitted and investigated in a suburban hospital The only positive finding was polycythaemia The facial palsy resolved gradually within 3 weeks and he was discharged from the hospital He was readmitted 3 months later into the same hospital following a new episode of generalized convulsions with a left peripheral facial palsy The patient was investigated again the polycythaemia was reconfirmed but it was also found that he had hypertension with blood pressure readings ranging from 180/110 to 270/1 0 mmHg He was then transferred to the University of Illinois Hospital

On examination the left facial palsy was obvious (Fig 1) The blood pressure checked repeatedly ranged between the levels mentioned above

Extensive investigations were carried out for his hypertension according to the schedule we practice in our department (1) The following investigations were performed Detailed physical examination and fundoscopy urinalysis and urine culture chest roentgenogram and electrocardiogram skull roentgenogram electroencephalogram and computerized axial tomography Urine concentration determinations and Addis count Complete blood cell count sodium potassium chloride glucose BUN creatinine β_1C PH bicarbonate cholesterol and triglycerides Lipoprotein electrophoresis and serum cortisol analysis Iodohippurate and technitium renal scans Twenty four hours urine collection for creatinine and urea clearance total protein excretion 17 ketosteroid and 17 hydroxycorticosteroid determinations Collection of urine for catecholamine quantification Peripheral-vein renin activity determination measurement of serum aldosterone electrolyte levels and aldosterone excretion rates in a 24 hours urine collection Contracted peripheral vein renin activity and selective renal vein renin activity were determined A percutaneous renal biopsy was per

DISCUSSION

Hypoglycaemia has been very rarely reported in association with 21 hydroxylase deficiency (4 6–9) which is not generally listed among the common causes of hypoglycaemia (1 5) Hypoglycaemic attacks occurred in only 2 out of 140 cases of adrenal hyperplasia followed up by Wilkins (8) According to Sauls & Ulstrom (6) congenital adrenal hyperplasia accounts for 4% of cases of spontaneous hypoglycaemia Job et al (2) reviewing the acute symptoms occurring over 6 years in 11 children with congenital 21 hydroxylase deficiency report no hypoglycaemic episodes In a review by Zuppinger (9) the occurrence of hypoglycaemia in children with adrenal hyperplasia is 1 40 Limbeck & Kelley (3) confirm the extremely infrequent reports of this complication which they expected to be more frequent considering the importance of cortisol in the maintenance of blood glucose Mackinnon & Grant (4) in 1977 reported two cases of 21 hydroxylase deficiency with hypoglycaemic seizures and emphasized that the risk of hypoglycaemia in this condition needs more accurate evaluation They suggested that this complication is perhaps more frequent than recorded in literature since it may at times be misinterpreted Their hypothesis seems to be confirmed by the history of our patient in whom hypoglycaemic convulsions occurred fairly frequently as a consequence of the stress of infection and in whom they were once wrongly attributed to epilepsy

REFERENCES

1 Ichiba Y & Bargman G J Hypoglycemia *In* L I Gardner (ed) *Endocrine and genetic diseases of childhood and adolescence* W B Saunders & Co Philadelphia 1975 p 931
2 Job J C Canlorbe P Lambertz J Courouble P & Rossier A Les accidents aigus au cours du traitement de l hyperplasie surrenale congenitale virilisante par défaut de 21 hydroxilase *Ann Pediatr 17* 476 1970
3 Limbeck G & Kelley V Congenital adrenal hyperplasia *In* V C Kelley (ed) *Metabolic endocrine and genetic disorders of children* Hagerstown (Maryland) 1974 p 265
4 Mackinnon J & Grant D B Hypoglycaemia in congenital adrenal hyperplasia *Arch Dis Child 52* 591 1977
5 Pagliara A S Karl I E Haymond M & Kipnis D M Hypoglycemia in infancy and childhood *J Pediatr 82* 365 1973
6 Sauls H S Jr & Ulstrom R A Hypoglycemia *In* V C Kelley (ed) *Brenneman s Practice of pediatrics* W F Prior Co Hagerstown Maryland 1966 vol I
7 White F P & Sutton L E Adrenogenital syndrome with associated episodes of hypoglycemia *J Clin Endocrinol 11* 1395 1951
8 Wilkins L *The diagnosis and treatment of endocrine disorders in childhood and adolescence* Thomas Springfield Ill 1965 3rd ed p 546
9 Zuppinger K A Hypoglycemia caused by diminished gluconeogenesis *In* K A Zuppinger (ed) *Hypoglycemia in childhood Evaluation of diagnostic procedures* S Karger A G Basel Switzerland 1975 p 102

Submitted May 17 1978
Accepted Sept 12 1978

(F D L)2 Clinica Pediatrica
Policlinico Universitario
98100 Messina
Italia

Acta Pædiatr Scand 68 287–289 1979

CASE REPORT

STRESS POLYCYTHAEMIA AND PERIPHERAL FACIAL PALSY COMPLICATIONS OF SEVERE HYPERTENSION

P M ZEIS S RAO E G JOHN and L C ASCHINBERG

From the Department of Paediatrics University of Illinois Hospital Chicago USA

ABSTRACT Zeis P M Rao S John E G and Aschinberg L C (Department of Paediatrics University of Illinois Hospital Chicago USA) Stress polycythaemia and peripheral facial palsy complications of severe hypertension Acta Paediatr Scand 68 287 1979.—An 11 month-old boy had an episode of generalized convulsions followed by a right peripheral facial palsy which resolved gradually within 3 weeks Three months later he had another similar episode of convulsions followed by a left peripheral facial palsy On both occasions it was found that he had polycythaemia A careful physical examination discovered that the child had severe hypertension Extensive laboratory investigations did not reveal a cause for his hypertension Haematologic investigations showed that the polycythaemia was due to a contracted plasma volume as a result of the hypertension The peripheral facial palsy most probably was due to a blood clot in the facial canal below the origin of the nerve to m stapedius as audiograms were normal and lacrimation preserved Control of the hypertension resulted in resolution of the facial palsy within 4 weeks and normal haematocrit readings within 6 weeks It should be stressed that every patient with peripheral facial palsy should be examined for hypertension

KEY WORDS Hypertension polycythaemia facial palsy

Polycythaemia has been reported in adults as an infrequent complication of hypertension (2) Another complication of severe hypertension more common in adults than in the paediatric age group is peripheral facial palsy (4 5)

We report a 14 month old boy with polycythaemia and peripheral facial palsy who was found to have severe hypertension

CASE REPORT

A 14 month-old boy was admitted to the University of Illinois Hospital with the diagnosis of malignant hypertension The boy was well until the age of 11 months when he had an episode of generalized convulsions followed by a right peripheral facial palsy for which he was admitted and investigated in a suburban hospital The only positive finding was polycythaemia The facial palsy resolved gradually within 3 weeks and he was discharged from the hospital He was readmitted 3 months later into the same hospital following a new episode of generalized convulsions with a left peripheral facial palsy The patient was investigated again the polycythaemia was confirmed but it was also found that he had hypertension with blood pressure readings ranging from 180/110 to 220/1 0 mmHg He was then transferred to the University of Illinois Hospital

On examination the left facial palsy was obvious (Fig 1) The blood pressure checked repeatedly ranged between the levels mentioned above

Extensive investigations were carried out for his hypertension according to the schedule we practice in our department (1) The following investigations were performed Detailed physical examination and [illegible] py urinalysis and urine culture chest roentgenogram and electrocardiogram skull roentgenogram [illegible] cephalogram and computerized axial tomography [illegible] concentration determinations and Addis [illegible] blood cell count sodium potassium chloride [illegible] BUN creatinine β C PH bicarbonate [illegible] triglycerides Lipoprotein electrophoresis [illegible] cortisol analysis Iodohippurate [illegible] scans Twenty four hours urine [illegible] and urea clearance for [illegible] and 17 hydroxycorticosteroid determinations [illegible] of urine for catecholamine [illegible] renin activity determinations [illegible] dosterone electrol[illegible] rates in a 24 hours urine [illegible] vein renin activity and [illegible] were determ[illegible]

Acta Pædiatr Scand 68 291–295 1979

CASE REPORT

THE ACIDIFICATION DEFECT IN THE SYNDROME OF RENAL TUBULAR ACIDOSIS WITH NERVE DEAFNESS

H SIMÓN B ORIVE I ZAMORA and S MENDIZABAL

From the Paediatric Nephrology Unit Hospital Infantil La Fe Valencia Spain

ABSTRACT Simon J Orive B Zamora I and Mendizabal S (Paediatric Nephrology Unit Hospital Infantil La Fe Valencia Spain) The acidification defect in the syndrome of renal tubular acidosis with nerve deafness Acta Paediatr Scand 68 291 1979 —An 8 year-old boy with renal tubular acidosis and nerve deafness has been followed for seven years Repeated studies of his renal acidification defect showed that until the age of six years the tubular defect was mixed proximal and distal (type 1 2 hybrid) After that age the defect of proximal acidification disappeared and the patient only presented a distal renal tubular acidosis type 1 When this is associated with nerve deafness it is considered a distinct nosological entity

KEY WORDS Renal tubular acidosis hereditary kidney disease nerve deafness nephrocalcinosis acid base balance

The syndrome of renal tubular acidosis (RTA) and nerve deafness has aroused interest over other associations of RTA and genetic diseases in the last decade The study of 18 cases described (3 6 13 20) or referred (5 12 14 17) suggest that we are dealing with a differentiated nosological entity with recessive autosomal genetic transmission The study of the tubular acidification defect has been documented only in three of the published cases (6 14) However all reports classify the RTA as distal or type 1 The retrospective study of the cases published by Cohen (19) has raised the doubt that one of them have a distal RTA with bicarbonate wasting or RTA type 3

This paper presents the follow up of a one year-old male affected by the syndrome of RTA and nerve deafness The renal acidification defect was initially diagnosed as a mixed RTA or type 1 2 hybrid At the age of seven the proximal defect disappeared while the distal RTA continues This evolutive aspect of the tubulopathy not referred to in the RTA nerve deafness syndrome could be related to studies of Saphira (18) on carbonic anhydrase activity

CASE REPORT

J P C male consanguineous born at term after a normal pregnancy and delivery Birth weight 3600 g At 13 months of age he was admitted to the hospital for persistent vomiting and failure to thrive (weight and height below the 3rd percentile) Blood studies were pH 7 19 P_{CO_2} 4 kPa sodium 141 mmol/l potassium 4 5 mmol/l chloride 114 mmol/l calcium 2 24 mmol/l phosphorus 1 6 mmol/l creatinine 44 micromol/l Calciuria was always higher than 0 25 mmol/kg/d urinary pH were between 7 and 8 Early morning urinary specific gravity after subcutaneous pitressin 1 011 In the urine protein and glucose were absent Blood and urine aminoacid screening were normal Radiological examination showed bilateral nephrocalcinosis A diagnosis of renal tubular acidosis was made and 6 5 mmol/kg/d of oral sodium bicarbonate were needed to maintain normal acid base status At 4 years of age because of speech delay hearing was tested The audiogram showed bilateral nerve deafness (Fig 2)

Family study The double consanguinity of the parents is shown in Fig 1 Neither deafness nor nephrolithiasis was detected in the family history In both parents and brother urinary acidification calciuria and urinary concentration were investigated All studies were normal

Table 1 *Ammonium chloride loading test*

	Patient age (years)	Plasma pH	Plasma HCO (mmol/l)	Urine (micromol/min/1 73 m) pH	TA	UV $_{H_4}$	UV_{HCO_3}	UV_H	$\frac{C_{CO_3}}{C_i} \times 100$
	3 10/1?	7 ?6	11	7 ?					
Study 1	5 ?/1?	7 ?6	11 7	7 30			53 6		3 8
Study	7 7/1	7 ?1	10 8	7 0	4 6	?9 7	5 0	−17 7	3 3
Normal values Mean (S D)					5? (33–71)	73 (46–100)	0	1.5	0

Normal values according to Edelmann et al (7)

Renal study of acid base equilibrium Tests of acidification were performed at ages of 3 10/12 years 5 2/12 years and 7 7/12 years They showed a consistent urinary acidification defect with a urinary pH always higher than 7 even at maximal acidemia (Table 1) Reabsorption of bicarbonate was tested at ages of 5 2/12 years (study 1) and 7 7/12 years (study 2) as shown in Fig 4 Both studies showed a constant plateau of bicarbonate excretion under 4% of the filtrate from levels of maximum acidemia to plasma bicarbonate concentrations of 15 mmol/l In study 1 (open signs) from this plasma bicarbonate concentration excretion abruptly increased reaching 27% of the filtrate at normal plasma levels In study 2 (closed signs) the plateau of bicarbonate excretion was consistently below 4% of the filtrate from levels of maximum acidemia to normal plasma concentrations of 20 mmol/l

Tubular reabsorption of sodium Tubular reabsorption of sodium during the hypotonic saline diuresis was checked at ages of 5 10/12 years (study 1) and 7 7/12 years (study 2) The data in *Table 3 correspond to the period of* minimum urinary osmolality (U $_m$) and maximum free water clearance (C_{H_2O}) when the activity of the antidiuretic hormone is minimal or absent $C_{H_2O}+C_{Na}$ could at this moment be an approximate indication of the amount of sodium delivery to distal tubule escaping proximal reabsorption Urinary volume represents the filtrate volume that reaches Henle s loop In study 1 a marked increase of $C_{H_2O}+C_{Na}$ (22 ml/100 ml GFR) was noted coinciding with the proximal loss of bicarbonate In study 2 the $C_{H_2O}+C_{Na}$ decreased to 14 ml/100 ml GFR simultaneously to the observed normalization in the proximal reabsorption of bicarbonate

The data outlined of C_{in} (137 and 128 ml/min/1 73 m²) RTP (84% and 91%) and Tm_{PO_4} (3 4 and 3 9 mg/100 ml GFR) were obtained prior to expansion with hypotonic saline infusion

Table 2 *Bicarbonate titration studies*

	Patient age (years)	Plasma pH	Plasma HCO (mmol/l)	$\frac{UV}{C} \times 100$ (micromol/100 ml GFR)	$\frac{C_{CO_3^-}}{C} \times 100$
Study 1	5 /1?	7 6	11 7	46 3	3 8
		7 44	0 0	538 6	6 9
Study	7 7/1	7 ?1	10 8	35 4	3 3
		7 46	0 3	97 8	4 8

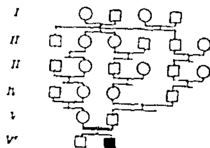


Fig 1 The pedigree of the kindred

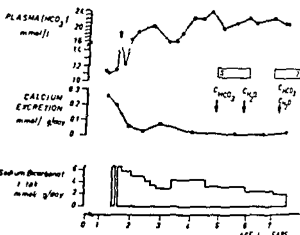


Fig 3 Clinical course Biochemical features as influenced by alkali therapy

METHODS

Glomerular filtration rate (GFR) was estimated by inulin clearance (C_i) according to the standard method. Tubular reabsorption of phosphorus (RTP) and its maximum tubular transport (Tm_{PO_4}) were calculated according to Bijvoet's nomogram (1).

Urinary excretion of hydrogen ion. An oral dose of 6 g/M² SA of ammonium chloride was administered according to Edelmann et al (7). During the next five hours plasma bicarbonate, urinary pH, titratable acidity (TA), ammonium excretion (UV_{NH_4}) and net acid excretion (UV_H) were determined.

Bicarbonate titration studies. Assessment of tubular reabsorption of bicarbonate was performed according to the procedure described by Edelmann et al (8). Special care was taken to minimize extracellular volume expansion keeping the bicarbonate infusion at a constant rate lower than 1 ml/min/m² SA.

Renal control of sodium and dilution ability. After an oral water overload in the preceding hour (70 ml/kg b w) a 0.45% hypotonic saline infusion was begun (2). At a rate of 4 ml/min/m² SA plus the urinary volume the total amount administered was 2000 ml/1 73 m

Analytic procedure. Inulin concentration was determined by the anthrone method of Davidson & Sackner (4), phosphorus according to the Fiske & Subbarow method (9). Blood pH and carbon dioxide content were measured in a Radiometer ABL 1. The urines were processed immediately after collection and the carbon dioxide was determined by a Natelson microgasometer. Urinary bicarbonate concentration was calculated from urinary pH and carbon dioxide content by the Henderson Hasselbach equation. pK was taken as $6.33-0.5\sqrt{(Na^{+})+(K^{+})}$ (10). Titratable acidity, ammonium and net acid excretion were determined according to the method of Jorgensen in an automatic Radiometer Titrator TTT 2 (11). O molality was estimated in an Advanced Osmometer 3D. Sodium and potassium were read on a IL 343 photometer.

RESULTS

The patient follow up over a period of seven years is shown in Fig 3. Acid base status and calcium levels were kept within normal range; the need of oral bicarbonate progressively lessened. At present at the age of 7 10/12 years weight and height percentiles are 97 and 90 respectively. Nephrocalcinosis is not increased. Renal function tests performed are shown in Fig 3.

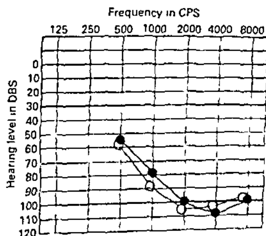


Fig 2 Patient's audiogram

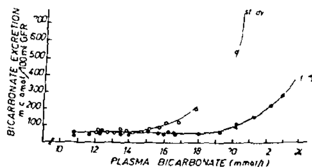


Fig 4 Excretion of filtered bicarbonate during continuous bicarbonate infusion. The time of titration studies is de

The syndrome of renal tubular acidosis with nerve deafness Acta Paediatr Scand 65 100 1976
7 Edelmann C M Boichis H Rodriguez Soriano J & Stark H The renal response of children to acute ammonium chloride acidosis Pediatr Res 1 45? 1967
8 Edelmann C M Rodriguez Soriano J Boichis H Gruskin A B & Acosta M I Renal bicarbonate reabsorption and hydrogen ion excretion in normal infants J Clin Invest 46 1309 1967
9 Fiske C H & Subbarow J The colorimetric determination of phosphorus J Biol Chem 66 375 19 5
10 Hastings A B & Sendroy J Jr The effect of variation in ionic strength on the apparent first and second dissociation constants of carbonic acid J Biol Chem 65 445 19_5
11 Jorgensen K Titrimetric determination of the net excretion of acid base in urine J Clin Lab Invest 9 87 1957
1 Koniesmark B W Renal tubular acidosis with progressive nerve deafness Personal communication cited by McKusick V A in Mendelian inheritance in man Johns Hopkins Press 1971 3rd ed p 506
13 Nance W E & Sweeney A Evidence for autosomal recessive inheritance of the syndrome of renal tubular acidosis with deafness Birth Defects Original Articles 7 70 1971
14 Nash M A Torrado A D Greifer I Spitzer A & Edelmann C M Tubular acidosis in infants and children J Pediatr 80 738 197
15 Oetliker O & Rossi E The influence of extracellular fluid volume on the renal bicarbonate threshold Pediatr Res 3 140 1969
16 Rodriguez Soriano J Vallo A & Garcia Fuentes M Distal renal tubular acidosis in infancy A bicarbonate wasting state J Pediatr 86 524 1975
17 Royer P & Broyer M L acidose renal au cours des tubulopathies congenitales In J Hamburger J Crosnier & J L Funck Bretano (eds) Actualites nephrologiques de l'Hopital Necker Flammarion Paris 1967 p 73
18 Saphira E Ben Yoseph Y Eyal F G & Russell A Enzymatically inactive red cell carbonic anhydrase B in a family with renal tubular acidosis J Clin Invest 53 59 1974
19 Sebastian A McSherry E & Morris R C Metabolic acidosis with special reference to the renal acidoses In B M Brenner & F C Rector (eds) The Kidney W B Saunders Philadelphia 1976 p 615
0 Walker W G Ozer F L & Whelton A Syndrome of perceptive deafness and renal tubular acidosis Birth Defects Orig Artic Ser 163 1974

Submitted March 6 1978
Accepted July ?1 1978

(J S) Paediatric Nephrology Unit
Hospital Infantil La Fe
Alferez Provisional ?1
Valencia 9
Spain

Table 3 *Data from maximal free water clearance and minimal urinosmolality during hypotonic saline diuresis*

	Patient age (years)	C_{In} (ml/min/ 1.73 m²)	RTP (%)	ml/100 ml GFR					
				V	U_{osm}	C_{osm}	C_{Na}	C_{H_2O}	$C_{H_2O}+C_{Na}$
Study 1	5 10/12	137	84	21.8	50	4	4.2	17.8	22
Study 2	7 7/12	128	88	15.1	76	4.1	3.1	11	14.1
Normal values[b]									
Mean		123		14.5	65	3.4	2.5	11.1	13.6
(S.D.)		(14)		(2.1)	(12)	(0.8)	(0.7)	(1.5)	(1.9)

[a] C_{In} and RTP values prior to hypotonic saline diuresis
[b] Normal values in a control group of 9 children

DISCUSSION

Reports of RTA nerve deafness syndrome always refer the acidification defect as a type 1 RTA. In this patient, when the first study of bicarbonate reabsorption was performed (study 1) a mixed RTA or type 1-2 hybrid was observed (19). When the study was repeated two years later (study 2) the proximal component had disappeared and the tubular defect had become an RTA type 1. Neither the abrupt increase of bicarbonate excretion observed in study 1 nor the patient's age suggest a distal RTA with bicarbonate wasting or type 3 RTA described in infants (16). The normal handling of phosphate (RTP, Tm) excludes hyperparathyroidism which could reduce proximal bicarbonate reabsorption. Two complementary facts in the patient follow up support the transformation of a mixed RTA (proximal and distal) to a classic distal RTA: 1) During the hypotonic saline expansion, the excessive sodium delivery to Henle's loop (study 1) and its later normalization (study 2); 2) The progressive reduction in alkaline therapy needed to maintain normal acid base status (Fig. 3).

According to experiences of Saphira (18) a transient lack of intraluminal carbonic anhydrase activity could be considered the cause of the proximal component showed in this patient.

Early diagnosis and treatment in this patient have allowed normal growth. Hearing loss diagnosed when he was 4 years old was not modified. This has caused a major delay in speech and social development necessitating attendance in a rehabilitation center. Late diagnosis of the hearing defect could be the cause of dumbness presented in three of the cases referred to by Dechaux (5). Donckerwolcke (6) claims attention to the necessity of revising hearing in all patients affected by distal or type 1 RTA.

ACKNOWLEDGEMENT

The authors thank Miss Visitacion Bartolomé, Miss Loreto Perez and Mr L. Rodado for their excellent technical assistance in performing the studies.

REFERENCES

1. Bijvoet O L M & van der Sluys Veer J. The interpretation of laboratory tests in bone disease. *Clin Endocrinol Metabol 1*: 217, 1972.
2. Chaimovitz C, Levi J, Better O S, Oslander L & Benderli A. Studies on the site of renal salt loss in a patient with Bartter's syndrome. *Pediatr Res 7*: 89, 1973.
3. Cohen T, Brand Aurabin A, Karshai C, Jacob A, Gay I, Tsitsianov J, Saphiro T, Jatzvi S & Ashkenazi A. Familial infantile renal tubular acidosis and congenital nerve deafness: an autosomal recessive syndrome. *Clin Genet 4*: 275, 1973.
4. Davidson W D & Sackner M A. Simplification of the anthron method for the determination of inulin in clearance studies. *J Lab Clin Med 62*: 351, 1963.
5. Dechaux M. Acidosis tubulaires primitives. *In* P Royer (ed.) *Seminaire de Nephrologie Pediatrique*. Paris 1977 (abstract) p. 45.
6. Donckerwolcke R A, Van Biervliet J P, Koorevaar G

NEW BOOKS RECEIVED

J F Burke & G Y Hildi k Smith (eds) *The infection p ne h sp tal patient* 5 pp Little Brown and Company Boston 19 8 $16 50 ISBN 0-316-11680 7

Neil Gordon *Pae li itri icu ol cy f the clinician* Clinical Developmental Medicine No 59/60 Spastics International Medical Publications 80 pp illus William Heinemann Medical Books Ltd London 1976

W Plenert & J Hermann *Ana mie i im Kind salter* Reihe moderne Padiatrie 144 pp illus VEB Georg Thieme Leipzig 1977 DM 35 —

D M Reed & F J Stanley *The epi lemic logy f pre n atu its* 370pp illu Urban & Schwarzenberg Munchen 1977 $18.50 ISBN 3 541 71611 8

J M Tanner *Foetus i to i i i* 50pp illus Open Books Publishing Ltd London 1978 £4 50 ISBN 0-7 91 0084 7

P B Beeson & D A Bass *The eosi ophil* Holt Saunders Ltd Eastbourne 1978 £10 50 ISBN 0 7216-1650-X

P L Morselli (ed) *Drug disp sition during devel pment* 490pp John Wiley & Sons Ltd Sussex 1978 £28 70/ $50 00 ISBN 0-89335 006-0

W E Bell & W F McCormick *Increased intracranial p essu e in children* nd ed *In* Major Problems in Clinical Pediatrics vol VIII 47_pp illus W B Saunders Company Philadelphia London Toronto 1978 £ 1 00 hardback ISBN 0-7216 1708 5

J V Simone (Guest editor) *Acute lei kaemi i In* Clinics in Haematology June 1978 00 pp illu tabl W B Saunders Company Ltd £16 50

M King F King & S Martodipoero *Primary ch ld care* A manual for health workers 315pp illus Oxford University Press Oxford 1978 £ 00 ISBN 0-19 647 9-4

Mark A Stewert & Ann Gath *Psychological dis rders f children* A handbook for primary care physicians 167 pp Williams & Wilkins Company Baltimore 1978 £10 95 ISBN 0-683 97179 9

tioned Michael Medici and Richard Gatti describe the normal host defence mechanisms as well as defects in neonates in an e sily comprehensible and highly informative way Some of the data they ha e collected are difficult to find in other books The reference list comprises 141 items Vincent Fulginiti reviews infections in children with immune defects For the pediatrician the last mentioned two sections are the most valuable in the book

Some papers include original investigations e g chapter 9 on host defences in protein calorie malnutrition by Robert Suskind and co-worker and chapter 10 written by Gerald Bodey and co-workers describing prevention of infection in patients with acute leukemia

The last part of the book (pp 157– 4) describing infections in surgi al patients is of minor interest for the pediatrician Changes in host defences in the immunosuppressed patient after kidney transplantation and prevention of deep wound infection following total hip replacement are reported as well as infections in surgical cancer patients

Readers seeking basic information on immune mechanisms in infectious diseases will find this in a more comprehensible way in ordinary textbooks of immunology But for those physicians who diagnose and treat infections in the compromised host the book is valuable as an account of the state of knowledge in this field Many of the data collected here are indeed difficult to find in other books

Anita Hallberg

J M Tanner *Foetus into man* Open Books Publishing Ltd London 1978 250 pp illus paperback £4 50 ISBN 0- 91-0084 *i*

This book deals with the fas inating and complex process of human growth from conception to maturity The well known author has in an admirable way succeeded to describe the different aspects and problems of growth in such a way that the book can be read by people without biological knowledge and still be a highly enjoyable reading for biologically trained professionals It is written in a clear and elegant often amusing style well illustrated and with a bibliography consisting of lists of further reading and of references The book contains twelve chapters beginning with the curve of growth and ending with disorders of growth Sex differentiation puberty developmental age endocrine control of growth etc are presented in different chapters For paediatricians this book is of great interest as a very comprehensive and easily read summary of what we know about growth to-day but it can also be highly recommended to all interested in human biology

C G Bergstrand

A Moragas A Ballabriga & M T Vidal *Atlas of ne natal hist pathol gy* W B Saunders Co Philadelphia London Toronto 1977 30 pp illus £43 75 ISBN 0-7 16-654 X

This translation of a Spanish edition from 1974 is an excellent atlas of neonatal pathology It is of great value for both paediatricians and general pathologists It is essentially an unusually well illustrated handbook composed of illustrative cases of mostly fatal neonatal conditions during the first month of life Brief clinical summaries accompany detailed histopathological descriptions referring to 3–4 illu trations in full colour on adjacent plates The photomicrographs are so well selected that the accompanying text is sufficient for comprehension without arrows

The book is based on an impressive series of 3 000 neonatal cases collected in a 7 year period in Barcelona All the classical lesions of the newborn baby are illustrated b th cl nically and morphologically

Mu h pa e is of course devoted to the respiratory sy tem (58 pp) including IRDS vascular lesions various infections and types of aspiration The liver (33 pp) kidney (3 pp) and heart (0 pp) are fully treated while rather small sections are concerned with the CNS the GI tract and the genitalia However some space is devoted to all other organs

Consequently this will be a standard reference book in the study of neonatal pathology mainly autopsy wise Surgical specimens are occasionally mentioned e g in a brief section on congenital tumours and on the liver

All books have their omissions This one has left out the largest of the baby s organs i e the placenta I would have liked it to be as well treated as all other organ systems Only a few references are mentioned in the text and are not accompanied by a bibliography

It was really a find to get the coup e Valdes–Dapena for the translation Together they master both Spanish and paediatric pathology Also Marie has extra experience in the field since she published a neonatal histology atlas (in 1957 in black and white) Their pleasure in undertaking the task is evident on every page The publishers are to be congratulated

The high price of this atlas must be weighed again t its high quality and persisting value It treats classical lesions that we all have to deal with on all continents for many years to come

Bio n Ivemark

BOOK REVIEWS

Neil Gordon *Paediatric neurology for the clinician* Clinics in Developmental Medicine No 59/60 Spastics International Medical Publications William Heinemann Medical Books Ltd London 1976 280 pp illus

Neil Gordon has addressed his book to the paediatrician who is not going to practice paediatric neurology himself His rationale for writing the book is the estimation that a third of all paediatric consultations involve hazards to the nervous system The author s aim has been to cover all aspects of paediatric neurology with the exception of those specific for the neonatal period

The overall impression of this book is that it reflects the author s vast clinical experience also the author is very willing to present his personal opinion The organization of the very rich material is at a first glance slightly confusing but turns out to be rather practical The wealth of details varies between chapters again obviously as a result of the author s varying personal interest

Paediatric neurology although it does not always provide any ready made discussions on differential diagnoses or details on pharmaceutical treatment can be recommended to any paediatrician because of its broad minded presentation good illustrations and adequate lists of references

Birgitta Jalling

J de Grouchy & C Turleau *Clinical atlas of human chromosomes* John Wiley & Son New York 1977 319 pp illus £24 70 ISBN 0-471 01704 3

The rapid development in cytogenetics since the introduction of the various chromosome banding techniques had led in recent years to an explosive and to the general pediatrician bewildering advance in the knowledge of chromosomal pathology Much of the new information is scattered in the literature and not always easy to find Now two distinguished cytogeneticists have brought it together in an admirable monograph

The Atlas has 23 chapters and 6 appendices One chapter is devoted to each chromosome of the human karyotype from chromosome No 1 to a short summary a description with illustrations of the morphology and banding pattern obtained by different techniques the gene map i e the genes assigned to this chromosome and information on the evolution of the chromosome in man and the other hominoid primates i e our closest cousins the chimpanzee the gorilla and the orang utan The most important part of each chapter however centers on the pathology presenting under separate headings the clinical features of old and new syndromes Each syndrome is illustrated by photographs of patients totally 464 photographs References including titles appear at the end of every syndrome presentation The appendices include a description of current cytogenetic techniques the basic concepts of palmar and digital dermatoglyphics terms used in clinical descriptions chromosomal nomenclature a list in alphabetical order of the genes located on the different chromosomes and a syndrome finder which classifies the most important symptoms and the syndromes they suggest

The material is well presented and easy to read The chromosomes and the syndromes for which they are responsible are described in a clear and concise way The illustrations are with few exceptions excellent In general the important bibliographies have been included and the references are as complete as can be expected up to 1976 My only criticism concerns Appendix III which is intended to list terms used in clinical description The presentation here is short and shallow The reader is however well rewarded by the wealth of information contained in the rest of the book

This book is highly recommended for pediatricians clinical cytogeneticists and other physicians interested in the relationship between chromosomal aberrations and congenital malformations It is the reviewer s wish that this Atlas will be kept up to-date every 2 years

Felix Mitelman

John F Burke and Gavin Y Hildick Smith (eds) *The infection prone hospital patient* Little Brown and Company Boston 1978 252 pp $16 50 ISBN 0 316 11680-7

The book consists of sixteen papers presented at a symposium devoted to the infection prone patient

The first chapter written by Paul Quie describes the role of polymorphonuclear neutrophils Then follows a paper by Zanvil Cohn who gives a conspectus of the role of macrophages in infectious processes Sir Gustav Nossal gives a short introduction to the cellular organization of the immune system and has chosen to concentrate on B cell function Data on the role of antibody in resistance to infection with *Pseudomonas aeruginosa* are given by Starkey Davis together with an account of *Pseudomonas* vaccines

Arthur Gottlieb gives a review of transfer factor in paper 5 Chapter 6 by Wesley Alexander and co-workers has the title Detection of defects in host defence mechanisms and their repair in the infection prone patient It is a bit surprising that tests for T cell defects are described without the sheep erythrocyte rosetting test being men

Acta Pædiatr Scand 68 301–303 1979

PROGENY OF SURVIVORS OF ACUTE LYMPHOCYTIC LEUKEMIA

P J MOE M LETHINEN R WEGELIUS S FRIMAN
A KREUGER and A BERG

From the Department of Paediatrics University of Tromsø Norway Section of Hematology Tampere Central Hospital Finland Department of Paediatrics Aurora Hospital Helsinki Finland Department of Paediatrics Lapin Hospital Rovaniemi Finland Department of Paediatrics University of Uppsala Sweden Department of Paediatrics Central Hospital Karlstad Sweden

ABSTRACT Moe P J Lethinen M Wegelius R Friman S Kreuger A and Berg A (Department of Paediatrics University of Tromsø Norway Section of Hematology Tampere Central Hospital Finland Department of Paediatrics Lapin Hospital Rovaniemi Finland Department of Paediatrics University of Uppsala Sweden Department of Paediatrics Central Hospital Karlstad Sweden) Progeny of survivors of acute lymphocytic leukemia Acta Paediatr Scand 68 301 1979.—Eight successful pregnancies and one spontaneous abortion have been observed in 5 women belonging to a group of 212 Nordic children who had their antileukemic therapy discontinued before January 1 1978 Furthermore a young leukemic man was the father of a healthy child after 4 years of intensive cytostatic therapy No malformations have been observed in the progeny of these treated individuals

KEY WORDS Acute lymphocytic leukemia cessation successful pregnancies offsprings

There have been dramatic advances in the treatment of acute leukemia in childhood during the past decade Following prolonged remission it has been possible to discontinue therapy in a large number of cases of acute lymphocytic leukemia in the Nordic countries (6) Subsequently only about 20% of 212 cases have relapsed after cessation of therapy (unpublished data) The long disease free period in many of these patients and other published cases justify the contention that cure is now possible in acute lymphocytic leukemia in childhood

Since prolonged remission or probably cure occurs in most treated cases of acute lymphocytic leukemia attention has been turned to the quality of their survival One of the important questions in that regard is whether the reproductive function of those patients is normal There are a few isolated reports of children born to survivors of acute lymphocytic leukemia and the incidence of defects in the offspring is not known (1 2 7 8) The purpose of this presentation is to report on 8 successful pregnancies in 5 women belonging to the Nordic group of 212 leukemic children who had therapy discontinued prior to January 1978 and a young leukemic man who became the father of a healthy child after 4 years of intensive cytostatic therapy including 2 years of central nervous system therapy

A survey is given of the material in Table 1

CASE REPORTS

Case 1

This female was born on March 10 1947 Acute lymphocytic leukemia (ALL) was diagnosed in March 1959 when she was 12 years old Induction therapy consisted of prednisone 90–100 mg daily and methotrexate 7 5 mg daily and maintenance therapy with 6 mercaptopurin (6-M P) 75–75 mg daily She first became pregnant in 1965 She discontinued the 6-M P medication after about one month of pregnancy three months later she told us about her pregnancy and it was decided to let her continue the medication The pregnancy was uneventful Her baby a healthy girl weighing 2760 g was born on December 17 1965 6-M P was discontinued in December 1965 after almost 7 years of antileukemic therapy Her second child a boy weighing 2850 g was born in April 1968 and her third child a girl weighing 3100 g was

ANNOUNCEMENTS

XVI INTERNATIONAL CONGRESS OF PEDIATRICS

The XVI International Congress of Pediatrics will be held in Madrid from the 8th–13th (inclusive) of September 1980 Information can be obtained from the following address Apartado de Correos No 29036 Barcelona Spain

The 7th European Congress of Perinatal Medicine be held in Madrid from the 14th–17th (inclusive) of S tember 1980 Information can be obtained from the foll ing address Apartado de Correos No 29015 Barcel Spain

INTERNATIONAL PRIZE FOR MODERN NUTRITION

The Central Association of Swiss milk producers announces the competition for the international prize in modern nutrition which it is donating for 1980 The prize amounts to SFr 15 000 — and will be awarded to a scientist from a member state of the international dairy farming association (Australia Belgium Brazil FRG Denmark Finland France Great Britain India Ireland Israel Italy Japan Canada Kenya Luxemburg Malta New Zealand The Netherlands Norway Austria Poland Sweden Switzerland Spain South Africa Czechoslovakia USSR)

The topic for 1980 is *Nutrition and Brain Devel pm* Deadline February 15th 1980 Authors of articles dea with the topic in question are invited to submit the fol ing documents in triplicate curriculum vitae bi graphy offprints of the most relevant articles relate the prize topic which he/she has had published du the past 5 years The documents should be writte either German French or English and submitted to President of the jury address Prof Dr H Aebi Me nisch chemisches Institut der Universitat Bern CH Bern 9 Switzerland

INTERNATIONAL SYMPOSIUM ON INFANT NUTRITION

An international symposium on infant nutrition and the development of the gastrointestinal tract will be held June 3–8 1979 at Niagara Hilton Niagara Falls New York Inquiries Continuing Medical Education (dren s Hospital 219 Bryant Street Buffalo New Y 14222 USA

EUROPEAN SOCIETY FOR SOCIAL PEDIATRICS

The Second International Congress of Social Paediatrics will be held in cooperation with the Turkish International Children s Center in Ankara June 7–9 1979 The main theme will be Childhood accidents—epidemiology and prevention Deadline for registration is April ? I Inquiries Prof Munnever Bertan Hacettepe Univer Ankara Turkey

Acta Pædiatr Scand 68 301-303 1979

PROGENY OF SURVIVORS OF ACUTE LYMPHOCYTIC LEUKEMIA

P I MOE M LETHINEN R WEGELIUS S FRIMAN
A KREUGER and A BERG

From the Department of Paediatrics University of Tromsø Norway Section of Hematology Tampere Central Hospital Finland Department of Paediatrics Aurora Hospital Helsinki Finland Department of Paediatrics Lapin Hospital Rovaniemi Finland Department of Paediatrics University of Uppsala Sweden Department of Paediatrics Central Hospital Karlstad Sweden

ABSTRACT Moe P J Lethinen M Wegelius R Friman S Kreuger A and Berg A (Department of Paediatrics University of Tromsø Norway Section of Hematology Tampere Central Hospital Finland Department of Paediatrics Lapin Hospital Rovaniemi Finland Department of Paediatrics University of Uppsala Sweden Department of Paediatrics Central Hospital Karlstad Sweden) Progeny of survivors of acute lymphocytic leukemia Acta Paediatr Scand 68 301 1979 —Eight successful pregnancies and one spontaneous abortion have been observed in 5 women belonging to a group of 212 Nordic children who had their antileukemic therapy discontinued before January 1 1978 Furthermore a young leukemic man was the father of a healthy child after 4 years of intensive cytostatic therapy No malformations have been observed in the progeny of these treated individuals

KEY WORDS Acute lymphocytic leukemia cessation successful pregnancies offsprings

ere have been dramatic advances in the atment of acute leukemia in childhood dur the past decade Following prolonged re ssion it has been possible to discontinue rapy in a large number of cases of acute nphocytic leukemia in the Nordic countries

Subsequently only about 20% of 212 cases ve relapsed after cessation of therapy (un blished data) The long disease free period many of these patients and other published ses justify the contention that cure is now ossible in acute lymphocytic leukemia in ildhood

Since prolonged remission or probably cure ccurs in most treated cases of acute lympho ytic leukemia attention has been turned to he quality of their survival One of the im ortant questions in that regard is whether the eproductive function of those patients is nor nal There are a few isolated reports of chil lren born to survivors of acute lymphocytic eukemia and the incidence of defects in the offspring is not known (1 2 7 8) The pur pose of this presentation is to report on 8 successful pregnancies in 5 women belonging to the Nordic group of 212 leukemic children who had therapy discontinued prior to January 1978 and a young leukemic man who became the father of a healthy child after 4 years of intensive cytostatic therapy including 2 years of central nervous system therapy

A survey is given of the material in Table 1

0 7991

CASE REPORTS

Case 1

This female was born on March 10 1947 Acute lymphocytic leukemia (ALL) was diagnosed in March 1959 when she was 12 years old Induction therapy consisted of prednisone 90–100 mg daily and methotrexate 2 5 mg daily and maintenance therapy with 6 mercaptopurin (6-M P) 75–75 mg daily She first became pregnant in 1965 She discontinued the 6-M P medication after about one month of pregnancy three months later she told us about her pregnancy and it was decided to let her continue the medication The pregnancy was uneventful Her baby a healthy girl weighing 2760 g was born on December 17 1965 6-M P was discontinued in December 1965 after almost 7 years of antileukemic therapy Her second child a boy weighing ?850 g was born in April 1968 and her third child a girl weighing 3100 g was

Table 1 *Survey of the material*

Case no	Sex	No of children	Abortion	Age at diagnosis (y)	Duration of antileukemic therapy (y)
1	F	3		12	6½
2	F	1	1	12	10
3	F	2		7	10
4	F	1		10	4½
5	F	1		15	5½
6	M	1*		21	6½ (until death)
Total		9	1		

The mother on therapy at the time of the first conception
* The father on therapy at conception

born in September 1973 All her 3 children are normally developed and in good health This case has been reported previously (8)

Case 2

A female was born on February 2 1955 ALL was diagnosed January 19 1968 Induction therapy consisted of prednisone 60 mg daily and maintenance therapy of 6-M P 125 mg daily until April 1969 when she relapsed Treatment with a combination of vincristine 2 mg weekly and prednisone 60 mg daily was started and a complete remission was achieved again Maintenance treatment with 45 mg methotrexate twice weekly was initiated in April 1969 Methotrexate was discontinued in September 1970 due to pulmonary infiltrations and 6-M P therapy 125–50 mg was reinstituted Antileukemic therapy was stopped in January 1976 after 10 years of cytostatic therapy In March 1977 the patient gave birth to a healthy girl weighing 4380 g and both the mother and child have later been completely well

Case 3

A female was born on April 13 1956 ALL was diagnosed in October 1963 Induction therapy consisted of prednisone 80 mg daily and 6-M P 75 mg daily and maintenance therapy of 6-M P 50–100 mg daily After 10 years of cytostatic therapy treatment was discontinued in October 1973 Her first child a boy weighing 2910 g was born in 1974 about one year after cessation of therapy and her second child a boy weighing 3070 g was born in 1976 Both children are healthy and normally developed

Case 4

A female was born on October 6 1962 ALL was diagnosed in January 1972 Induction therapy consisted of prednisone 60 mg daily 6-M P 75 mg daily and vincristine 2 mg once weekly and maintenance therapy of 6-M P 75 mg daily The maintenance treatment was decreased to 75 mg five times weekly on March 1972 One year later cranio-spinal irradiation with 2200 rad was given Antileukemic therapy was discontinued in September 1976 She became pregnant in January 1977 and gave birth to a healthy boy weighing 3460 g in October 1977 The mother and child have been in good health since

Case 5

A female was born on September 3 1953 ALL was diagnosed in December 1968 Induction therapy was initiated with prednisone 90 mg daily and 6-M P 100 mg daily Maintenance therapy consisted of 6-M P 100 mg daily All therapy was discontinued in March 1974 She had a spontaneous abortion in second month in February 1976 In August 1977 she gave birth to a boy weighing 3190 g He had slight calcaneo-valgus deviation of both feet Both the mother and the son are otherwise well

Case 6

A male born on October 1 1948 ALL was diagnosed in August 1970 Induction therapy with prednisone 80 mg daily and vincristine 4 mg once weekly was initiated and he received maintenance therapy with 6-M P 150 mg–100 mg daily Central nervous leukemia was diagnosed in January 1973 and he received intensive therapy with methotrexate intrathecally He was also in 1973 given 3 courses of COAP (cytosine arabinoside 150 mg daily prednisone 200 mg daily cyclophosphamide 200 mg daily for 5 days and vincristine 2 mg once) Conception took place while he was on therapy He married and had a two months prematurely born daughter in December 1975 weighing 2100 g Blood group studies indicated that the patient was the father of the child The patient was continued on therapy until he died in March 1977 from hematological relapse 6½ years after therapy was initiated His daughter has developed normally and is currently healthy

DISCUSSION

Now when cure is possible in children with ALL attention must be focused on the late effect of antileukemic therapy An important question to these young patients is what prospects of reproduction they have and the possible effects of different types of antileukemic therapy upon the development of the fetus

The children in long term remission from acute leukemia seem to have a good chance of normal development of puberty particularly those diagnosed in early childhood (7) There are however reported only a few cases of off spring of survivors of acute childhood leuke mia (1 2 7 8) We have now reported 9 off springs (now 1–13 years old) of 6 survivors of ALL all of whom are normal Only one of the ten pregnancies ended with spontaneous abor tion We do not know however whether abor tion has occurred in any of the other survivors of ALL A study reported by Li & Jaffe on the progeny of 45 childhood cancer survivors showed no excess of children with defects (5) That study included however only two cases with leukemia

The five females received a relatively con servative type of treatment regimen with moderate intensive induction and only one drug as maintenance None of our cases re ceiving high doses of methotrexate have so far become pregnant Case 4 received cranio spinal irradiation Our male patient had re ceived intensive cytostatic therapy including high dose of methotrexate and three courses of COAP (including cyclophosphamide) be fore the conception It is usual in the Nordic countries today to discontinue antileukemic therapy after 3 years of continuous remission Most cases of leukemia in childhood occur before the age of ten years Children above 13 years are in fact high risk cases with a small chance of long term survival The problem of offsprings of such cases off therapy will there fore be rare even in the future We must how ever calculate on numerous pregnancies in children who have terminated their anti leukemic therapy here in the Nordic coun tries in recent years (6)

It seems important to collect data on all pregnancies in long term leukemia survivors and try to obtain information on their off springs This study is too limited in number to permit any sweeping conclusions but the initial results are reassuring More studies are required before any definite conclusion can be drawn both as to long term prognosis in leuke mia survivors and the possible effects of the more intensive cytostatic treatment regimens on pregnancy

It is too early to ascertain whether central nervous system irradiation or the use of high dose methotrexate will cause fetal damage

More data are needed concerning the long term effects of the disease and the effects of different types of cytostatic therapy both on the patients and their offsprings There are some data which indicate that close to 20% of children receiving irradiation for malig nancy will develop a second malignancy with in 20 years (5) It is however too early to calculate the risk of secondary malignancy in children receiving intensive cytostatic therapy for acute leukemia Finally nothing is known as to whether offsprings of leukemic survivors have increased risk of neoplasia Answers to these vexing questions will be apparent only after these treated patients and their children have been under surveillance for many years

REFERENCES

1 Bacon C & Kernahan J Successful pregnancy in acute leukemia *Lancet II* 515 1975
Editorial The price of survival in childhood leukemia *Br Med J I* 371 1978
3 Li F P Second malignant tumors after cancer in childhood *Cancer 40* 1899 1977
4 Li F Cassody J R & Jaffe N Risk of second tumors in survivors of childhood cancer *Cancer 35* 1 30 1975
5 Li F F & Jaffe N Progeny of childhood cancer survivors *Lancet II* 707 1974
6 Moe P J Cessation of therapy in childhood leuke mia A survey of a Nordic material consisting of 16 cases *Acta Paediatr Scand 67* 145 1978
7 Sins E S Leventhal B G & Vaitukaitis J L Effects of childhood leukemia and chemotherapy o puberty and reproductive function in girls N *Eng J Med* 94 1143 1976
8 Wegelius R Successful pregnancy in acute leukemia *Lancet II* 1301 1975

Submitted Nov 23 1978
Accepted Dec 29 1978

(P J M) Department of Paediatrics
University of Trondheim
Trondheim
Norway

Acta Pædiatr Scand 68 305–313 1979

QUALITY OF CARE A TRACER DIAGNOSIS STUDY OF ACUTE OTITIS MEDIA COMPARING A DISTRICT PAEDIATRIC SERVICE WITH PAEDIATRIC AND OTOLARYNGOLOGY EMERGENCY DEPARTMENTS

P OLIN P BOLME G EWERT B LAGERKVIST G STERKY
K TENGVALD and R ZETTERSTRÖM

From the Department of Paediatrics St Goran s Hospital the Graduate School of Social Work and Public Administration and the ENT Department Sabbatsberg s Hospital Stockholm Sweden

ABSTRACT Olin P Bolme P Ewert G Lagerkvist B Sterky G and Zetterstrom R (Department of Paediatrics St Goran s Hospital Stockholm Sweden) Quality of care A tracer diagnosis study of acute otitis media comparing a district paediatric service with paediatric and otolaryngology emergency departments Acta Paediatr Scand 68 305 1979 Acute otitis media was used as a tracer condition for comparing patient care as regards patient satisfaction compliance and medical outcome in a district paediatric office an otolaryngological and a paediatric hospital emergency department The initial work up of the 216 patients studied at the three units was in good agreement with predefined standards However while the district paediatrician arranged a follow up for all patients the hospitals did so only in 10–30% of the visits Parent satisfaction was significantly higher at the district office when the time spent waiting and the time the patient was with the doctor were kept constant Urinary penicillin was not detected in 1/17 of district paediatric and in 6/19 of otological patients Compliance with follow up was also much higher at the district paediatrician Medical outcome did not differ between the units The majority of the parents expressed preference for the type of care given in a neighbourhood service It is suggested that the criteria for adequate treatment of acute otitis media adapted for emergency departments may be revised in case easy access is provided to a medical centre offering continuity of care and proper follow-up Studies of the quality of care have so far not managed to demonstrate a definite and positive relationship between the process of care patient satisfaction and final medical results

KEY WORDS Quality of care otitis media district paediatric service

The concept of quality of care is poorly defined and the variables that are included in such studies differ considerably among different authors (7 9 22 23 26) The process of medical care may be evaluated by measuring medical outcome and patient satisfaction Brook & Appel (6) related the long term outcome for specific diagnoses to the type of care given as compared with established criteria for diagnosis and treatment They showed that the outcome was poorly related to adherence to established medical norms Becker et al (2) showed that continuity of care in a hospital setting was associated with higher parent satisfaction and better compliance In primary care where patients often follow courses of treatment prescribed by the medical team positive patient or parent satisfaction may be necessary to achieve compliance and adequate medical outcome Patient satisfaction may also be considered as a goal in its own right since medical services are expected to satisfy the requirements of patients These are some reasons why patient satisfaction and for paediatric practice parent satisfaction is regarded as an important measure of the quality of care (2 14)

The aim of this study is to compare the quality of care in a district paediatric service with hospital facilities This report is part of an evaluation of a district paediatric service with integrated health and sick care for a defined child population in a suburb of Stockholm (13)

Table 1 *Summary of issues studied and type of data used*

Issues	Data
Structure of medical service	
i e training working hours waiting time etc	Questionnaire to physicians
Content of medical care	
Diagnostic procedures compared to professional standard	Questionnaire to physicians
Measures and recommended follow up	Questionnaire to physicians
	Questionnaire to parents
Parental perception of care	
Satisfaction	Questionnaire to parents
	Interview with parents 5 months later
Family compliance with	
Medication	*Urinary penicillin determination*
Follow up	Chart review
	Contact with the physicians
	Interview with parents 5 months later
Medical outcome	
Number of visits within 5 months	Chart review
Hearing test	Play audiogram
Social background	Questionnaire to parents

We used a tracer diagnosis design (12) Acute otitis media was selected as the tracer condition since approximately 20 to 25% of first visits to the district paediatrician and 6% to the paediatric emergency department were for treatment of this condition (13) In addition this diagnosis is well defined The beneficial effect of adequate treatment was considered well established and final outcome easily determined (12)

We collected information on the following factors (Table 1) the structure and content of medical services the parents satisfaction and compliance with the regime and the final medical outcome in order to study how the organization of medical services influences the quality of care given Background data on patient characteristics were also obtained in order to assess selective demands for medical services

MATERIAL

Medical services studied

The comprehensive district paediatric service was situated in a newly built suburb about 12 km from the

Table 2 *Patients studied at three clinics*

Medical unit	Patients studied	Parent questionnaire	Telephone interview	Urinary penicillin	Audiogram[b]
District paediatrician	56	48	46	17	15
Paediatric emergency department	93	77	77	–	11
Otolaryngological emergency department	67	60	59	19	41
Total	216	185 (86%)	182 (84%)	36	67

[a] Urinary penicillin determined in children older than 3½ years within an accessible geographic area The three children at the paediatric hospital who were eligible were not included in the analysis Nine children at the district paediatrician s office and five at the otological hospital were not reached in time for the urine sample Two otolaryngological patients refused

[b] Pure tone audiograms were performed on children older than 3½ years Eleven were not reached and two refused to participate

Table 3 *Demografic and social data of the families*

Variable		District paediatrician		Paediatric emergency department		Otolaryngology emergency department	
		N	%	N	%	N	%
Age of child		56		93		67	
	<1 years		16		30		8
	1–3 years		43		50		30
	>3 years		41		20		63
Marital status of mother		46		73		59	
	Married or cohabitant		93		88		83
	Single		7		12		17
Age of mother		45		74		58	
	<20 years		0		4		3
	20–29 years		69		64		52
	>29 years		31		32		45
Educational level of mother (school years)		48		75		58	
	>12		6		11		10
	10–12		56		44		35
	<10		38		45		55
Working hours of mother		47		71		59	
	Fulltime		23		23		44
	Part time (1–35 hrs/week)		55		39		31
	Not employed		21		38		25
Social class (father)		43		67		46	
	I		19		38		22
	II		23		2		13
	III		54		54		59
	Not classified		4		6		6
Tenure		46		75		59	
	Rental		59		61		64
	Owner occupied		41		39		36
Bedroom standard		46		73		59	
	≤1 person/room		54		58		66
	1–2 persons/room		46		41		33
	>2 persons/room		0		1		0

centre of Stockholm. The office was staffed by a paediatrician (Board certified 1971) and a paediatric registered nurse and was open four hours per day Mondays through Fridays. The paediatrician was working in the adjacent child health centre and was the school physician for the same population during the remaining office hours on weekdays. All families were living within walking distance of the paediatric service. After office hours emergency care was available at the two hospitals studied.

The otolaryngological emergency department is located in a general hospital in the centre of Stockholm. The paediatric emergency unit belongs to a university department located in another hospital. Both are open 24 hours a day. Emergency care is primarily given by several physicians in postgraduate training. Physicians and auxiliary staff work in the emergency room on a rotating schedule. It is therefore impossible to provide continuity of care in most instances.

General sickness insurance covers the visits to all clinics studied. The fee was 15 Sw. kr. per visit.

In the end of September 1974 data collection was started in the office of the district paediatrician for two weeks followed by one month of simultaneous recording at all three services and concluded by three weeks of collection at the district paediatric service. The extended time compensated for the smaller catchment area of the neighbourhood service.

Patients studied

Table 2 shows the number of patients participating in the study as described below under *Methods*.

According to the background data obtained from the questionnaires answered by parents some differences in family characteristics were found between the units (Table 3). The paediatric emergency department was more frequently visited by children less than one year old. The mothers were often not working outside the home. The otolaryngological department saw more often older children with older working mothers. The families visiting the district paediatrician were intermediate fo

Table 4 Criteria for diagnosis of acute otitis media

The diagnoses were used in the following order purulent simplex non specific otitis otosalpingitis when physical findings within several categories were noted

	Otitis			
	Purulent	Simplex	Non specific	Otosalpingitis
History				
Ear discharge	×			
Acute ear ache no positive physical finding			×	
Physical findings				
Ear discharge	×			
Drum abnormalities				
Perforation	×			
Bulging	×			
Red		×		
Dull and thickened		×		
Red malleus		×		
Retracted				×
Clear fluid in the middle ear				×
Decreased movement				×
Unspecified abnormalities			×	
Sore tragus			×	

several variables Thus the district paediatrician served as an alternative to both emergency departments In the following analysis we will only compare the district paediatrician with the two hospital units and refrain from matching the latter The socioeconomic differences did not seem to influence our main findings They show however that the type of clinic used is selected to some extent to suit the mother s working conditions

METHODS

We agree upon criteria for the diagnosis treatment and follow up of acute otitis media before the data collection started this was in accordance with Swedish and international studies (1 3 19 24) and local oto laryngology praxis Acute otitis media according to our criteria may be divided into purulent otitis simplex otitis and non specific otitis (Table 4) The table also includes the criteria for otosalpingitis

The initial care was considered adequate when at least ten days of treatment with antibiotics in combination with nasal and/or oral decongestive agents were prescribed and a follow up visit was arranged In case of oto salpingitis decongestive therapy and arrangements for follow up sufficed (3)

Data concerning diagnosis and initial treatment were collected with questionnaires to the participating physicians at the visit Participating physicians were informed about the aim of the study and asked to complete a questionnaire for each child aged six months to 15 years with acute otitis media or otosalpingitis according to his/her own assessment (13) No more than three symptoms and three signs supporting the diagnosis were to be included in the questionnaire in addition to the diagnostic tests used drugs prescribed and follow-up arrangements made Data on the duration of the visit and the patient s general condition were also collected

The parents were given written information about the study at the time of the visit and were asked to participate and to answer a questionnaire mailed to their homes within five days (13) Background factors about the patient parent preference and satisfaction were derived from this questionnaire and from a telephone interview with the parents five months later

Compliance was checked by chart reviews regarding follow up visits and in some instances by determination of penicillin in the urine during treatment Urine samples for control of treatment were obtained by a unannounced telephone call from children over two and one half years of age and living in the suburbs close to the district paediatrician The samples were collected at home within one hour frozen and later analyzed for penicillin by a microbiological technique (5 11)

At the telephone interview five months after the visit the parents were questioned about the child s current condition and hearing The response rate for the parent questionnaire and interview was 82–90% at the different units (Table 2) A major reason for non participation was language difficulties The study was not aimed at the specific problems of ethnic minorities To elucidate such questions other methods should be used The remaining nonparticipants included the abstaining parents and comprised less than 10% of the material

The number of medical contact made because of ea trouble since the index visit and complications such a chronic otitis serous otitis and remaining perforation o an otological operation or adenoidectomy were recorde by chart reviews after six months Children older than 3 years were offered an audiometric examination five t six months after the visit

After coding crosstabulation and statistical calculatio were performed employing an IBM 60 programme (18) χ^2 values corresponding to $p<0.05$ were considere statistically significant and $p<0.001$ were highly statisti cally significant

RESULTS

Medical process

Most children had a simplex otitis according to our criteria (Tables 4 and 5) The proportion of children with purulent otitis was of the same magnitude in the district paediatrician s office and the otolaryngological emergency room The general condition of the children was rated fair to good by the physicians at all

Table 5 *Patients with otitis at different clinics subgrouped according to criteria in Table 1*

Medical unit	Otitis			Otosal pingitis (%)	Not classifiable (%)	Total	
	Purulent (%)	Simplex (%)	Nonspecific (%)			(%)	N
District paediatrician	27	64	7	0	2	100	56
Paediatric emergency department	17	75	5	0	2	99	93
Otolaryngological emergency department	8	63	5	3	2	101	67
Total	–3	68	6	1	2	100	216

clinics There was no evidence that more serious illnesses were selectively seen by otologists Most patients (90% of the 210 with otitis) were prescribed antibiotics for ten days Of the 24 children who were not given antibiotics none had purulent otitis according to our criteria Treatment was similar at all three units

The district paediatrician arranged follow up visits for all of her patient at her office

The otolaryngological emergency department arranged follow up visits at the hospital for 30% and at the paediatric emergency department for less than 10% Seventy percent of the patients at both hospitals were advised to contact another physician

Parent preferences

In the questionnaire the parents were asked to select between pairs of mutually exclusive alternatives of medical service By this technique different aspects of the neighbourhood and hospital services were juxtaposed such as continuity of care versus proximity to consulting experts easy access to daytime service versus 24 hour service and proximity to home versus hospital resources About half of the families wanted a neighbourhood medical service with the same doctor supplemented by a 24 hour emergency facility However 50% of those who had experience of the district paediatrician thought it possible to wait 24 to 48 hours before consulting a doctor in the case of acute otitis—a condition which according to many otologists requires emergency treatment (Table 6)

Parent satisfaction

How did the expressed preference for the neighbourhood centre correspond to the parents attitudes towards the service given at the different units? The parents were

Table 6 *Parental preferences for the provision of care for acute otitis according to questionnaire*

N = number of parents responding to all relevant questions

Unit	Neighbourhood service (%)	Neighbourhood service + emergency room (%)	Hospital + continuity of care (%)	Hospital (%)	Other (%)	Undecided (%)	Total (%)	N
District paediatrician	50	40	0	0	0	10	100	40
Paediatric emergency department	5	47	8	4	8	9	100	57
Otolaryngology emergency department	9	57	15	2	11	6	100	44
Total	19	48	16	2	7	8	100	141

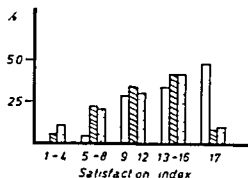


Fig 1 Parental satisfaction with the visit the doctor and auxiliary staff according to questionnaire and interview The index was based on ten questions regarding the parent s opinion of the visit in general and of the physician and nurse(s) performance with respect to contact with the child helpfulness information given and time spent A high score indicates high satisfaction Percentages for each unit Open bars=district paediatric visits (N=40) hatched bars=paediatric emergency visits (N=59) stippled bars=otolaryngological emergency visits (N=49)

much more often satisfied with the visit to the district paediatrician than the visits to hospitals This was also true of their appreciation of the doctor s management of the patient and the auxiliary staff's activity The answers to several questions regarding parental satisfaction were compiled into indexes for the three units (Figs 1 and 2) The higher satisfaction expressed for the service given by the district paediatrician could be due to various structural differences The waiting time there seldom exceeded one hour (5%) and was usually less than 20 min (65%) In the emergency departments a waiting time of one hour or more was common (34–54%) the longest waiting time being in the department of paediatrics Parents who had to wait more than one hour were less satisfied However variations in waiting time within one hour did not affect satisfaction at each unit

The district paediatrician spent slightly more time with her patients The doctors at the otolaryngological emergency department were quickest However there was no relation between the short time spent by the doctor and requests for more time or information The differences between the district paediatrician and the hospitals regarding satisfaction were still statistically significant when waiting time was below one hour and doctor time was 6–15 min This shows that other factors are involved in parental satisfaction

Patient compliance

Determinations of penicillin the urine on the fifth to the eighth day of treatment were performed in 19 children from the otolaryngological department and in 17 from the district paediatrician Six of the hospital children as compared to one of the district paediatrician s children had no measurable quantity of penicillin in the urine Thus 1/3 of the hospital children did not have adequate treatment as compared with 1/17 at the neighbourhood service The difference is significant at the 5% level using Fisher s exact test The difference could be related to the confidence in the district paediatrician s office as suggested by the analysis of parent satisfaction This was also illustrated by the district paediatric nurse s own explanation When a *mother phones and tells me that she* cannot give pencillin to her child I just tell her to come down and I ll show her how to do it

Follow up of otitis is often considered to be a necessary part of treatment in order to

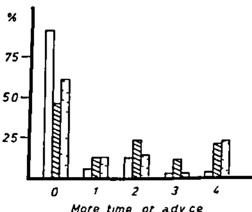


Fig 2 Parental requests for more time or advice at the visit according to the questionnaire The figures correspond to the number of requests made for more time or advice from either the physician or the nurse Percentages as in Fig 1 Distric paediatric visits (N=4?) paediatric emergency visits (N=45) otolaryngological emergency visits (N=47)

reduce the risk of future loss of hearing The chart review showed that 98% of the district paediatrician s children had a follow up as compared to only 54% of the otolaryngo logical and 42% of the paediatric hospital patients The differences between the district paediatrician and the hospitals were highly sig nificant Parent satisfaction within each clinic was not related to whether follow up was performed or not

Medical outcome

Eleven of the 93 paediatric emergency patients were referred to an otolaryngological depart ment or underwent an otological operation or adenoidectomy within six months Cor responding figures for the district paediatric patients were nine of 55 and for the oto laryngological emergency patients 14 of 62 The differences between the neighbourhood and the hospitals patients were not statisti cally significant However among patients attending a scheduled follow up visit the proportion of later complications was highest among the otolaryngological patients Twelve of 26 underwent an operation as compared to nine of 55 district paediatric ($p<0.01$) and six of 34 paediatric emergency patients Thus the paediatric patients received the same amount of specialist care irrespective of follow up The otolaryngological emergency department on the other hand had a larger proportion of patients with complications among those who made follow up visits The result may indicate that the otologists more ac curately selected the patients requiring follow up In addition the families may themselves compensate for inadequate follow up at the paediatric hospital

After five to six months two of 15 children at the district paediatric service showed a hearing loss of at least 25 decibels in either ear as compared with four of 11 paediatric hospital patients and five of 41 at the otolaryngological emergency unit The differ ences were not statistically significant The patient s satisfaction was not related to the medical result as measured by residual hearing loss or otological complications

GENERAL DISCUSSION

Families that attend emergency departments at hospitals prefer to some extent the type of care given by the district paediatrician The preference is more marked in persons who have visited the neighbourhood centre This result is in accordance with Skinner et al (20) and emphasizes the importance of direct referrals from the hospitals to the relevant neighbourhood centre

The diagnostic and therapeutic programme at the district paediatrician s office was adequate for the diagnosis studied and sur passed the hospitals by offering an adequate follow up

The neighbourhood service met many of the demands expressed by the parents Waiting time was shorter and more time was spent by the doctor Continuity of care was provided during office hours since the same physician and nurse examined the patients whereas in hospitals the same doctor seldom saw the patient at the infrequent follow up visits

The diagnostic precision and the initial treatment varied less than expected at the different clinics In a study of paediatric hospital care of gastroenteritis great differ ences between reasons for admission and duration of hospital stay were found (21) Similarly in studies initiated in response to the legislation of professional standards review organizations in the USA emphasis has been placed on showing the great varia tions in existing medical care rather than in demonstrating the deviation from a given professional norm (25) The conformity may mirror the uniform teaching in the depart ments of otolaryngology at medical schools and the efficiency of the postgraduate pro grammes in Sweden

However the criteria used for treatment of otitis media should be questioned Treatment

with antibiotics for ten days and the compulsory follow up visit may not be necessary in all cases of uncomplicated otitis provided easy access to a neighbourhood medical centre can be made available in case of complications Rigid adherence to specified medical norms adjusted to a system offering fragmented and non continuous medical care may prove unnecessary and costly where an efficient neighbourhood service is provided

This study demonstrates the deficiencies in care given at the emergency departments studied especially the disregard for the effect of discontinuity of care and unsatisfactory follow up routines on patient compliance The results are in agreement with several studies from emergency departments on children treated with penicillin for acute tonsillitis or otitis where 30–50% did not complete prescribed treatments according to various criteria (2 4 8 10) Earlier studies were focused on patient factors Any hypotheses that the variations in satisfaction and compliance reflect variations in social background knowledge about infections and level of expectation among the families at the three clinics are not confirmed by our data Rather the results are in accordance with the supposition that the local paediatric service was in a better position to carry out a regime than the hospital emergency units

More extensive studies of the quality of care although imperfect (15 16 17) are important means for clarifying weaknesses in the existing medical system and for suggesting alternative programmes for treatment in improved settings

REFERENCES

1 Armstrong B W The treatment of acute middle year infection in children *Otolaryngol Clin North Am 3* 249 1970
2 Becker M H Drachman R H & Kirscht J P Predicting mother s complicance with pediatric medical regimens *J Pediatr 81* 843 1972
3 Bergholtz L Allmanna principer for otitbehandling *In* R D Rudberg & H Hallander (eds) *Symposium over diagnostik och behandling av akut otit sinuit och tonsillit* Astra Lakemedel Sodertalje *1973 p 141*
4 Bergman A B & Werner R J Failure of children to receive penicillin by mouth *N Engl J Med 68* 1334 1963
5 Bolme P & Eriksson M Influence of diarrhea on the oral absorption of penicillin V and ampicillin in children *Scand J Infect Dis 7* 141 1975
6 Brook R H & Appel F A Quality of care assessment choosing a method for peer review *N Engl J Med 288* 1323 1973
7 Donabedian A Evaluating the quality of medical care *Milbank Mem Fund Q 44* no 3 166 1966
8 Elling R Whitmore R & Green M Patient participation in a pediatric program *J Health Hum Behav 1* 183 1960
9 De Geyndt W Five approaches for assessing the quality of care *Hospital Administration* Winter 1970 *21 1970*
10 Gordis L Markowitz M Lilienfeld A M The inaccuracy in using interviews to estimate patient reliability in taking medications at home *Med Care 7* no 2 49 1969
11 Jalling B Malmborg A S Lindman A & Boreus L O Evaluation of micromethod for determination of antibiotic concentrations in plasma *Eur J Clin Pharmacol 4* 150 1972
12 Kessner D M & Kalk C E *Contrasts in health status vol 2 A strategy for evaluating health services* Institute of Medicine National Academy of Sciences Washington D C 1973 p 11
13 Lagerkvist B Olin P Tengvald K Sterky G & Zetterstrom R *Distriktsbarnlakare Studier av vard utnyttjande och vardkvalitet i en storstadsforort Sprı rapport 11* 1976
14 Lebow J L Consumer assessments of the quality of medical care *Med Care 12* 328 1974
15 Lindsay M I Jr Hermans P E Nobrega F T & Ilstrup D M Quality of care assessment 1 Out patient mangement of acute bacterial cystitis as a model *Mayo Clin Proc 51* 307 1976
16 McNerney W J The quandary of quality assessment *N Engl J Med 295* 1505 1976
17 Morehead M A Ambulatory care review A neglected priority *Bull N Y Acad Med 52* 60 1976
18 Nie N H Hull C H Jenkins J G Steinbrenner K & Bent D H *Statistical package for the social sciences* SPSS New York 1975 2nd ed
19 Rudberg R D Acute otitis media Comparative therapeutic results of sulphonamide and penicillin administered in various forms *Acta Otolaryngol* Suppl 113 1 1954
20 Skinner T J Price B S Scott D W & Gorry G A Factors affecting the choice of hospital based ambulatory care by the urban poor *Am J Public Health 67* 439 1977
21 Smedby B & Sundelin C Admission rate and length of stay for nonspecific enteritis in pediatric departments in Sweden *Int J Health Serv 2* 537 1972

Starfield B & Scheff D Effectiveness of pediatric care The relationship between processes and out come *Pediatrics 49* 547 197?
3 Thorner R M Health program evaluation in re lation to health planning *Health Serv Rep 86* 525 1971
?4 US Dept Health Education and Welfare Hearing sensitivity and related medical findings among chil dren *Vital Health Stat 11* no 14 197?
?5 Welch C E Arena J M Cocco A Light F V Lucas F Porterfield J D Shalowitz M Slee V N & Steinholtz L H PSRO and norms of care *JAMA 229* 166 1974
6 WHO *Methods of evaluating public health programmes* Report on a symposium Kiel November 196? WHO Europ 0375 1968

Submitted May 30 1978
Accepted Oct 28 1978

(P O) Sachsska Barnsjukhuset
S 100 64 Stockholm
Sweden

with antibiotics for ten days and the compulsory follow up visit may not be necessary in all cases of uncomplicated otitis provided easy access to a neighbourhood medical centre can be made available in case of complications Rigid adherence to specified medical norms adjusted to a system offering fragmented and non continuous medical care may prove unnecessary and costly where an efficient neighbourhood service is provided

This study demonstrates the deficiencies in care given at the emergency departments studied especially the disregard for the effect of discontinuity of care and unsatisfactory follow up routines on patient compliance The results are in agreement with several studies from emergency departments on children treated with penicillin for acute tonsillitis or otitis where 30–50% did not complete prescribed treatments according to various criteria (2 4 8 10) Earlier studies were focused on patient factors Any hypotheses that the variations in satisfaction and compliance reflect variations in social background knowledge about infections and level of expectation among the families at the three clinics are not confirmed by our data Rather the results are in accordance with the supposition that the local paediatric service was in a better position to carry out a regime than the hospital emergency units

More extensive studies of the quality of care although imperfect (15 16 17) are important means for clarifying weaknesses in the existing medical system and for suggesting alternative programmes for treatment in improved settings

REFERENCES

1 Armstrong B W The treatment of acute middle year infection in children *Otolaryngol Clin North Am 3* 249 1970

2 Becker M H Drachman R H & Kirscht J P Predicting mother s compliance with pediatric medical regimens *J Pediatr 81* 843 1972

3 Bergholtz L Allmanna principer for oltbehandling *In* R D Rudberg & H Hallander (eds) *Symposium over diagnostik och behandling av akut otit sinuit och tonsillit* Astra Lakemedel Sodertalje 1973 p 141

4 Bergman A B & Werner R J Failure of children to receive penicillin by mouth *N Engl J Med* 268 1334 1963

5 Bolme P & Eriksson M Influence of diarrhea on the oral absorption of penicillin V and ampicil in children *Scand J Infect Dis 7* 141 1975

6 Brook R H & Appel F A Quality of care assessment choosing a method for peer review *N Engl J Med 288* 1323 1973

7 Donabedian A Evaluating the quality of medical care *Milbank Mem Fund Q 44* no 7 166 1966

8 Elling R Whitmore R & Green M Patient participation in a pediatric program *J Health Hum Behav 1* 183 1960

9 De Geyndt W Five approaches for assessing the quality of care *Hospital Administration* Winter 1970 21 1970

10 Gordis L Markowitz M Lilienfeld A M The inaccuracy in using interviews to estimate patient reliability in taking medications at home *Med Care 7* no 2 49 1969

11 Jalling B Malmborg A S Lindman A & Boreus L O Evaluation of micromethod for determination of antibiotic concentrations in plasma *Eur J Clin Pharmacol 4* 150 1972

12 Kessner D M & Kalk C E *Contrasts in health status vol 2 A strategy for evaluating health services* Institute of Medicine National Academy of Sciences Washington D C 1973 p 11

13 Lagerkvist B Olin P Tengvald K Sterky G & Zetterstrom R *Distriktsbarnlakare Studier av vard utnyttjande och vardkvalitet i en storstadsforort Spri rapport 11* 1976

14 Lebow J L Consumer assessments of the quality of medical care *Med Care 12* 328 1974

15 Lindsay M I Jr Hermans P E Nobrega F T & Ilstrup D M Quality of care assessment 1 Out patient mangement of acute bacterial cystitis as a model *Mayo Clin Proc 51* 307 1976

16 McNerney W J The quandary of quality assessment *N Engl J Med 295* 1505 1976

17 Morehead M A Ambulatory care review A neglected priority *Bull N Y Acad Med 52* 60 1976

18 Nie N H Hull C H Jenkins J G Steinbrenner K & Bent D H *Statistical package for the social sciences* SPSS New York 1975 2nd ed

19 Rudberg R D Acute otitis media Comparative therapeutic results of sulphonamide and penicillin administered in various forms *Acta Otolaryngol* Suppl 113 1 1954

20 Skinner T J Price B S Scott D W & Gorry G A Factors affecting the choice of hospital based ambulatory care by the urban poor *Am J Public Health 67* 439 1977

21 Smedby B & Sundelin C Admission rate and length of stay for nonspecific enteritis in pediatric departments in Sweden *Int J Health Serv 2* 537 1972

? Starfield B & Scheff D Effectiveness of pediatric care The relationship between processes and out come *Pediatrics 49* 547 197?

?3 Thorner R M Health program evaluation in relation to health planning *Health Serv Rep 86* 5?5 1971

4 US Dept Health Education and Welfare Hearing sensitivity and related medical findings among children *Vital Health Stat 11* no 14 197?

?5 Welch C E Arena J M Cocco A Light F V Lucas F Porterfield J D Shalowitz M Slee V N & Steinholtz L H PSRO and norms of care *JAMA 2?9* 166 1974

?6 WHO *Methods of evaluating public health programmes* Report on a symposium Kiel November 196 WHO Europ 0375 1968

Submitted May 30 1978
Accepted Oct ?8 1978

(P O) Sachsska Barnsjukhuset
S 100 64 Stockholm
Sweden

Acta Pædiatr Scand 68 315–322 1979

THE PROGNOSIS OF NEAR DROWNED CHILDREN

S KRUUS L BERGSTROM T SUUTARINEN[1] and R HYVONEN[2]

From the Research Department Rinnekoti Institution for the Mentally Retarded Espoo [1]Department of Neurology University of Helsinki and [2]Children s Hospital University of Helsinki Finland

ABSTRACT Kruus S Bergstrom L Suutarinen T and Hyvonen R (Research Department Rinnekoti Institution for the Mentally Retarded Espoo Department of Neurology University of Helsinki and Children s Hospital University of Helsinki Finland) The prognosis of near-drowned children Acta Paediatr Scand 68 315 1979.—Thirty children were treated for near-drowning in the Children s Hospital University of Helsinki during 1971–1976 The patients were divided into 3 groups according to the prognosis group I included 13 children (43%) with a favourable prognosis group II four children (13%) with a less favourable prognosis who developed severe sequelae and group III 13 children with poor prognosis and in whom the subsequent outcome proved fatal The surviving children underwent neurological neurophysiological and psychological examination 6–58 months after the accident The children in group I had slight neurological or psychological signs some children presented a lowered intellectual functioning level The children in group II were tetraplegic unable to speak and had convulsions The following factors were important in affecting prognosis the longer the immersion time the worse the prognosis However prognosis could still be favourable with an immersion-time of 11–20 min Prognosis was bad if the first pH value was <7 00 The arterial oxygen pressure values measured during the treatment did not correlate with the prognosis but a low rectal temperature on admission was usually associated with a bad prognosis The degree of EEG-disturbance had a prognostic value However the follow up recordings correlated better with the prognosis than the recordings during the first 24 hours after which worsening of the EEG sometimes showed a progressive brain lesion

KEY WORDS Near-drowning children

Drowning in Finland as in many other countries is one of the three leading causes of death in childhood Every year a number of near drowning victims are treated in children s hospitals The prognosis for a complete recovery is held to be very good (4 13) In the material of 36 near drowned children Eriksson et al found two seriously disabled cases In a series of 29 near drowned patients of all ages Modell (13) confirmed that there were no neurological deficits In the former study the sequelae were estimated by interviewing the parents in the latter study the main interest was concentrated on following pulmonary function after near drowning The aim of our study is to present the clinical findings in the acute period after the near drowning and to consider the prognosis of near drowned children using neurological neurophysiological and psychological examinations

During the progress of this work Modell et al (14) Fandel & Bancalari (5) and Pearn (16) published their material concerning the prognosis for near drowning

MATERIAL AND METHODS

The material consists of 30 children who were admitted to the Children s Hospital University of Helsinki because of near-drowning during 1971–1976 The age distribution was 7 months–13 years 11 months median 4 years 4 months Twenty three (77%) of them were boys seven (23%) girls (Table 1)

All the children had been submerged in water and consciousness had been lost Twenty patients were brought to the Children s Hospital directly following first aid the

Table 1 *Distribution of near drowned children according to age and sex*

Age (years)	Sex		
	Boys	Girls	Total
<1	2		2
1–4	10	4	14
5–9	7	3	10
10–14	4		4
Total	23	7	30

other ten were transferred from other hospitals within 20 hours of the accident

Eight of the children had been found in a lake seven in a ditch or pit seven in a swimming pool four in the sea three in a waterbucket and one in a river

The Baltic seawater near the Finnish coast contains a very small amount of sodium chloride (0 02 to 0 75%) The submersions in the sea happened near Helsinki Thus it could be said that all the accidents in this series belong to the category of freshwater-drownings

The information concerning the clinical course in the acute period was collected from hospital records All 17 children who survived were re examined The period from the accident to re investigation varied from 6–58 months (median 22 months mean 29 months) The re examination included an interview with the parents and an EEG recording as well as the clinical neurological examination and psychological testing

Psychological tests were performed on 13 children WISC (Wechsler Intelligence Scale for Children) was applied to 9 children TML (the Version of Terman Merril tests standardised in Finland) was chosen in three cases and WPPSI (Wechsler Preschool and Primary Scale for Intelligence) in one case To measure the visual motor coordination Bender was used in 11 cases and drawing simple geometric figures was used in the two youngest patients

The intelligence of the four most handicapped children was estimated by a child neurologist

An estimation of pre accident intellectual capacity was made from the interview with the parents In one case pre accident psychological testing was available

A total of 91 EEG recordings were performed on 28 patients Twenty patients were examined within 24 hours of the accident Following this 24 patients were examined between 24 hours to 2 weeks after the accident and 20 patients thereafter At the time of the first EEG recording some patients were being *artificially respirated*

The EEG patterns were classified according to Pampiglione (15) Class 1 includes the recordings in which the activity mainly corresponded to that of age limits or showed only slight slowing Class 2 includes the recordings in which the brain activity was mainly in the delta range Class 3 includes the recordings in which brain activity was composed of periods of irregular slow waves with or without spikes or sharp waves Between the periods of activity silent periods *lasting* a few or several seconds were obtained Class 4 includes isoelectric EEGs

RESULTS

The material has been divided into thre groups according to the subsequent outcom (Table 2) Group I includes children whos prognosis had been favourable (13 patient 43%) Group II includes those patients wi severe neurological sequelae (4 patient 13%) Group III consists of those childre who died in hospital (13 patients 43%)

Correlation of the immersion time to th outcome (Table 3) The exact immersio time was known only in one case In 18 case it could be estimated but in 11 cases estim tion was impossible The approximate imme sion time was 5 minutes in seven cases all which belonged to group I In one case hy poxia lasted 15 min longer than the immersio time because of a delay in beginning resusc tation

Four children were immersed for between to 10 min—in three of them the prognosis ha been favourable while one had severe seque

Table 2 *Appearance of neurological and psy chological sign in near drowned children*

P pre accident signs + slight ++ marked +++ se vere – no abnormal findings

Group	Pat	Neuro-logical findings	Psychological findings	
			Lowering of general IQ	Failure in visuomotor coordination
I	2	P	–	P
	7	+	+	++
	8	+	–	++
	9	+	–	–
	14	+	+	++
	16	P	–	P
	20	+	+	++
	21	–	–	+
	22	–	–	P
	24	–	–	+
	28		–	++
	29	–	–	P
	30	P	–	P[b]
II	1	P +++	+++	+++
	13	+++	+++	+++
	19	+++	+++	+++
	27	+++	+++	+++

Reading and writing disorder
[b] Spatial diffic

Table 3 *Immersion time according to prog nosis in near drowned children*

Immersion time (min)	Favour able prog nosis (Group I)	Severe sequelae (Group II)	Fatal out ome (Group III)	Total
≤5	7			7
6–10	3	1		4
11–20	3	1	4	8
No estimation		2	9	11
Total	13	4	13	30

In one case a delay in resuscitation of about 15 min

lae Eight children were immersed for 11 to 20 min Of these three had a favourable progno-sis although there were some signs of a brain lesion One patient had severe sequelae and four died

Of the 11 cases in which the immersion time could not be estimated all had a bad prognosis two ending with severe sequelae and nine with death

Clinical findings in the acute period (Table 4) In group I two patients did not need any resuscitation seven were resuscitated imme diately after being brought out of the water or on the way to hospital and in four cases re suscitation had to be continued in hospital In one case where the rectal temperature of the patient was 25°C the heart started to beat but arrested later and the patient had to be re resuscitated

Six patients in group I required mechanical ventilation and four of them had PEEP (po-sitive end expiratory pressure) The ventila tion lasted from 2 to 4 days except in two cases where a pneumothorax complicated the therapy and the ventilation was thus prolonged to eight days All patients in groups II and III needed mechanical ventilation In half of the cases PEEP was used In group II the dura tion of the ventilation varied from 4 to 9 days

Corticosteroid treatment was used in eight patients in group I and in all the patients in groups II and III Signs of pulmonary oedema were observed in the X ray examination of 27

Table 4 *Data concerning the patients with a favourable prognosis (group I) in the acute period and the neurological findings on re examination after near drowning*

Case	Age (years)	Immersion time (min)	Mechanical ventilation (days)	Return of conscious ness	Seizu res	Other neurolo gical signs in the acute period	Neurological findings in the re-examination
	3	10		At scene		Slight spasticity in all limbs co ordination failure absence fits	Same as in the acute period
7	6	0	*	6 h		Sluggish	–
8	1	10	1+8	21 d		Tetraplegia spastica	Coordination failure sluggish
9		5	3	4 d	+		Coordination failure
14	3	15	1+6*	10 d			Coordination failure Babinski + at right
16	13	5	4	1 d	+	Hypotonia musculorum	Coordination failure
0	5	15	3	3 d		*Hemiparesis levis* 1 dx nystagmus strabism	Same as in the acute period
1	9	10		5 h	+	Dystonia tetraplegica	Coordination failure athetoid movements
	11	5		At scene			Normal
4	5	5		3 h			Normal
8	8	3		At scene		–	Normal
19	9	3		At scene		–	Normal
30	8	3		At scene			Normal
1						Stumbling gait headache	Coordination failure spatial difficulties

Pre-accident signs * Delay in beginning resuscitation was about 15 min
Pneumothorax on nd day * Pneumothorax on 3rd day

Table 5 *Initial blood pH values according to prognosis in near drowned children*

Initial blood pH values	Favourable prognosis (Group I)	Severe sequelae (Group II)	Fatal outcome (Group III)	Total
<7 00		1	6	7
7 00–7 34	12	2	3	17
Unknown	1	1	4	6
Total	13	4	13	30

patients Three patients belonging to group I had no pulmonary oedema

The initial blood pH values taken within 2 hours of the accident are presented in Tab 5

Repeated arterial oxygen (Pa_{O_2}) measurements were made in most of the cases (Fig 1) Pa_{O_2}-values did not correlate with the prognosis

The first rectal temperature measurements on admission to the hospital are presented in Fig 2 The lowest value was 25°C Thirteen patients had rectal temperatures below 30 C—in all these cases the immersion time had been longer than 5 min and 11 patients were immersed during the cold season It seems that a low initial rectal temperature is often associated with a bad prognosis

Neurological state in the acute period (Table 4) Eight patients in group I were unconscious on admission Three patients returned to full consciousness on the same day and three within 4 days In two patients the period of unconsciousness was longer (10 and 21 days) and both suffered the complication of pneumothorax (cases 8 and 14) The patients in groups II and III were all unconscious on admission None of the patients in group III returned to full consciousness before death Many of the children had seizures during the acute period

Six patients in group I showed no neurological signs after consciousness returned The other seven had neurological symptoms two of them showing signs that had existed previously The short case reports of the five patients with neurological signs caused by immersion are as follows (Table 4) *Case 7* A 6-year old girl was very slow in her reactions and speech She has improved slightly *Case 8* An 18 month old boy had spastic tetraplegia two weeks after the accident After six weeks he began to walk and later to speak *Case 14* A 3 1/2 year old boy with the complication of a pneumothorax had marked muscle hypotonia and could not speak Four weeks later he could walk and speak but his muscle power was still not good *Case 20* A 5 1/2 year old boy still had a tendency to opistotonus severe dystonic tetraplegia and choreoathetosis four

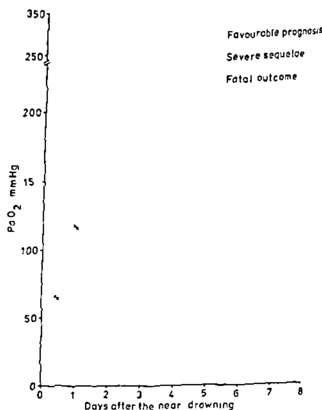


Fig 1 Arterial oxygen tension values in near drowned children



Fig 2 Rectal temperature on admission to the hospital and the prognosis of near drowned children

weeks after the accident He neither spoke nor could he fix his eyes Two months later he began to walk and speak and his vision was good *Case 30* A 8 year old boy was discharged home on the third day after the accident His gait was stumbling and he suffered severe headache on the fifth day after the accident when corticosteroid treatment was discontinued The headache lasted four days The gait was normal after five days

The neurological and psychological findings on re examination (Tables 2 and 4) On re examination five children out of 17 survivors had no neurological symptoms Five children in group I who had had neurological findings in the acute period still had slight neurological signs (coordination failure) at the time of re examination It appeared from the interview with the parents that three other patients in group I with neurological signs had already had the same neurological signs before the accident (cases 2 16 and 30)

In group I all the patients underwent psychological testing The median of the general IQ was 96 (mean 99 2 range 88 to 115 Fig 3) except for two children whose IQs were low (48 and 76 cases 2 and 16) and whose intelligence was already low before the accident In three cases the functioning level was estimated to be lower than before the accident These patients had marked specific difficulties and/or a lack of concentration

In the WISC all the other patients showed corresponding verbal and performance scores except for two in whom differences were seen In Bender s test seven children in group I had failure of visual motor coordination Failure was marked in five of them and slight in two

In group II all patients were mentally retarded and tetraplegic with difficulty in swallowing They could not move or speak All of them reacted to auditory stimuli and recognised their mothers voices Three did not react to visual stimuli but none had optic atrophy Three had seizures despite anticonvulsant medication All except one were living in hospitals One of these children had pre accident psychomotor retardation but had learned to walk before the accident

In group III ten patients died within ten days and three lived in a decerebrate state before dying of pneumonia within 8 months of the accident All three had severe cerebral necrosis on autopsy

EEG findings (Table 6) In group I eight patients did not show any abnormalities in the acute period or at re examination In five patients there was a slight general slowing in the EEG Two of them had a normal EEG recorded within 24 hours of the accident but the EEG recorded one week later showed moderate slowing One of them (case 30) complained of headache On re examination all patients in group I showed normal EEG activity except for one in whom paroxysmal activity was shown in the EEG 3 1/2 years later This patient had the complication of pneumothorax during the acute period

In group II two patients were examined

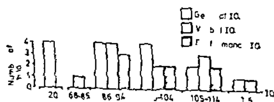


Fig 3 Intelligence levels in 15 near-drowned children The verbal and the performance IQ was measured in nine children

Table 5 *Initial blood pH values according to prognosis in near drowned children*

Initial blood pH values	Favourable prognosis (Group I)	Severe sequelae (Group II)	Fatal outcome (Group III)	Total
<7 00		1	6	7
7 00–7 34	12	2	3	17
Unknown	1	1	4	6
Total	13	4	13	30

patients Three patients belonging to group I had no pulmonary oedema

The initial blood pH values taken within 2 hours of the accident are presented in Tab 5

Repeated arterial oxygen (Pa_{O_2}) measurements were made in most of the cases (Fig 1) Pa_{O_2} values did not correlate with the prognosis

The first rectal temperature measurements on admission to the hospital are presented in Fig 2 The lowest value was 25°C Thirteen patients had rectal temperatures below 30°C—in all these cases the immersion time had been longer than 5 min and 11 patients were immersed during the cold season It seems that a low initial rectal temperature is often associated with a bad prognosis

Neurological state in the acute period (Table 4) Eight patients in group I were unconscious on admission Three patients returned to full consciousness on the same day and three within 4 days In two patients the period of unconsciousness was longer (10 and 21 days) and both suffered the complication of pneumothorax (cases 8 and 14) The patients in groups II and III were all unconscious on admission None of the patients in group III returned to full consciousness before death Many of the children had seizures during the acute period

Six patients in group I showed no neurological signs after consciousness returned The other seven had neurological symptoms two of them showing signs that had existed previously The short case reports of the five patients with neurological signs caused by immersion are as follows (Table 4) *Case 7* A 6-year old girl was very slow in her reactions and speech She has improved slightly *Case 8* An 18 month old boy had spastic tetraplegia two weeks after the accident After six weeks he began to walk and later to speak *Case 14* A 3 1/2 year old boy with the complication of a pneumothorax had marked muscle hypotonia and could not speak Four weeks later he could walk and speak but his muscle power was still not good *Case 20* A 5 1/2 year old boy still had a tendency to opistotonus severe dystonic tetraplegia and choreoathetosis four

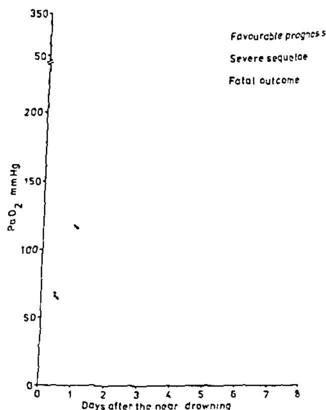


Fig 1 Arterial oxygen tension values in near drowned children

cellent and that 95% of the surviving children are neurologically normal

In this Finnish study the outcome was more often fatal and post accident neurological deficits were seen more often than in other materials Our patients were treated like Modell s (14) and Fandel s (5) patients after the principles evaluated by Modell (13) Modell points out that near drowning in fresh water affects pulmonary function Washing out of the surfactant factor leads to atelectasis and there are risks of pulmonary oedema bronchial obstruction pneumonitis and pneumonia In nearly all of our cases the arterial oxygen pressure could be held within a safe area and there was no block in ventilation pulmonary function or in the pulmonary circulation The brain lesion was the crucial factor In Pearn s series (16) the children were immersed in freshwater but the immersion times were much shorter than in this Finnish experience i e from 0 5 to 10 min (mean 3 7 min) In our material the immersion time could not be calculated exactly except in one case In addition after the children are brought out of the water there may have been delay in the beginning of resuscitation or resuscitation may not have been done correctly Both these factors lengthen the duration of an inadequate bloodsupply to the brain It has been suggested that submersion for longer than five minutes is uniformly fatal (11) Results in the monkey after one hour s complete experimental ischemia provide evidence that it may be possible to survive much longer immersion (7)

Cases of survival without sequelae have been reported after submersion for 17 20 22 and even 40 min (8 9 10 18) The cause of recovery in these cases was held to be due to hypothermia resulting from falling into cold water

In the Finnish material hypothermia was not a good prognostic sign Seventy percent of the patients with an initial rectal temperature of less than 30 C had a bad prognosis as opposed to 25% of the patients with a rectal temperature of 35°C or more It can be concluded that hypothermia like a low blood pH value indicates a long immersion time and more severe brain anoxia

There is little information regarding re warming to be found in the available literature From our experience active re warming should not be done quickly and should not exceed 32 C At this temperature the heart functions well but the brain has a reduced oxygen requirement The seizures seen in four patients with a favourable prognosis occured during active re warming indicating an increased need of the brain for oxygen (1 3)

In this study the first EEG recordings were of some prognostic value the EEGs of the patients who survived were normal or only slightly disturbed The recordings from the patients who died showed more pathological brain function

In the follow up the EEG s of the patients who had recovered had returned to normal despite having been initially disturbed In the patients with severe sequelae or who died the EEG had worsened In many cases the worsening appeared in the form of paroxysms However the initial worsening of the EEG was not always indicative of severe neuro-psychological sequelae In two patients there had been a transient general slowing but in the follow up period the EEG had returned to normal The clinical symptom of the slowing period of the EEG was headache At this time no disturbances in the lung function could be detected Thus some other mechanism other than late lung oedema must be the cause of this phenomenon

On the basis of our experience we think that EEG evaluation is a very useful method for assessing the prognosis of near-drowning The follow up recordings correlated better with the prognosis than did recordings within 24 hours of the accident after which time a worsening of brain function could still occur This is inconsistent with findings on the prognostic value of the EEG in brain anoxia after cardiocirculatory arrest where the rate of

Table 6 *EEG changes (1–4 see text) in various prognostic groups correlated with the time interval after near drowning*

Prognostic groups	EEG classes Time after near drowning 24 hours	1≤14 days	>14 days
Group I	1	1	1
Favourable	1	1	1
prognosis	1	1	1
		1	1
		1	1
		1	1
		1	1
			1
	1	2	1
	1	2	1
	2	2	1
	2	2	1
		2	1
Group II	1	2	1
		2	1
Severe	1	2	2
sequelae		2	3
Group III	1	1	4
Fatal outcome	1		
	2	2	3
	2	4	
	2	4	
	3		
	3	4	
	3	2	
	4	4	
	4	4	
	4		

With paroxysms

within 24 hours of the accident and both of them showed normal brain activity However within two weeks a worsening of condition had occurred leading to a class 2 EEG Two other patients were examined for the first time during the week following the accident Both of them were classified as class 2 On re examination more than one month after the ac cident the EEG s of all the patients in group II were pathological Three had paroxysmal activity and one showed a flat EEG

In group III 11 patients were examined within 24 hours of the accident Six of them had an EEG pattern which was classified as class 3 and 4 and the other five patients were classified as 1 and 2 In all patients the EEG became progressively worse or showed no re covery except in one patient who died fou months later

DISCUSSION

Two factors affecting accident proneness in childhood are raised by this study The first is one of sex Boys were victims in 77% of the cases same tendency being found in other childhood accidents (12 19) Biological factors and upbringing make boys more active than girls Even small boys are expected to be un dependent and to play alone while girls are more restricted by being held closer to the mother (6)

The other factor affecting accident pro neness would appear to be neuropsycholog cal According to the parents six of the sur viving 17 children had some preaccident neu ropsychological sign Three children had sub normal intelligence with neurological signs Two of them had difficulty in reading and writing and one had spatial difficulties Fail ure in perception and motor coordination make such children unable to cope with dangerous situations

According to this study the prognosis after near drowning is favourable when the immer sion time does not exceed 10 min the initial blood pH value is above 7 00 and the patient regains consciousness fairly rapidly i e with in a few days In the acute period they may show marked neurological symptoms which resolve Their EEG recordings are normal or slightly altered

In this study 56% of the patients survived The prognosis was favourable in 43% and 13% of the patients had severe neurological seque lae The outcome was fatal in 43% of the cases In Modell s series (14) of 91 near drowning patients 89% survived and two pa tients had neurological deficits In Fandel s and Bancalari s material (5) 82% of the chil dren survived 12% had severe neurological deficits findings which are the same as in our material Pearn (16) maintained that the prog nosis of near drowning in childhood is ex

Acta Pædiatr Scand 68 323–328 1979

CLINICAL COURSE OF WHOOPING COUGH IN CHILDREN YOUNGER THAN SIX MONTHS

B TROLLFORS

From the Department of Infectious Diseases Ostra Sjukhuset Goteborg Sweden

ABSTRACT Trollfors B (Department of Infectious Diseases Östra Sjukhuset Goteborg Sweden) Clinical course of whooping cough in children younger than six months Acta Paediatr Scand 68 323 1979 —The patient records of 59 children aged 2–26 weeks with culture verified pertussis were analysed Twenty four of them were hospitalized in most cases for social reasons Only one child with hypothyroidism and a complicating pneumonia was critically ill Seventeen of the 35 non hospitalized patients had a mild disease without developing typical whooping attacks Thirteen children were treated with erythromycin in the catarrhal stage There was a tendency towards milder disease in this group but the differences compared to untreated children were not statistically significant

KEY WORDS Pertussis infants

Despite a high immunization rate whooping cough is still a common disease in many countries In Sweden there has been an impression among clinicians that the disease has become milder during the last two decades even though it has become more common This is also indicated by a study by Strangert (5) From the United Kingdom Miller & Fletcher) have reported that pertussis is still a serious isease in children under six months but has a benign course in older patients As children aged under six months seem to have more severe disease this study was undertaken to evaluate the severity of the disease in this age group

PATIENTS AND METHODS

At the Department of Clinical Bacteriology in Gothenburg Sweden which serves an area of about 700 000 inhabitants *B pertussis* was isolated in nasopharyngeal cultures from 1 630 patients between January 1 and December 31 1977 Sixty four of them were children aged below six months Patient records were available for study in 59 of these cases (29 boys 31 girls) The children had been seen at the University Hospital in Gothenburg two smaller hospitals (Mölndal and Uddevalla) and nine pediatric outpatient clinics When the patient records were incomplete additional information was obtained from the parents

RESULTS

Hospitalization The frequency and reasons for hospitalization in different age groups are summarized in Table 1 The 24 hospitalized children had a mean period of hospital stay of 7 5 days (range 1–24 days) If four patients with longer stay for other reasons (hypothyroidism serious social problems) are excluded the mean duration of hospital stay was somewhat less than five days The child with the complicating pneumonia (8 weeks[1]) was the most critically ill He was hospitalized one week after the beginning of the whooping attacks with extensive pulmonary infiltrates For the first 24 hours he was kept in an oxygen tent After two days he was already markedly improved but stayed in hospital for four weeks as he had a congenital hypothyroidism and a suspected cardiac lesion requiring further investigation

Two of the children admitted because of apnoea and cyanosis improved quickly and were discharged after two and five days respectively The third child in this group had a

[1] Age when symptoms started

EEG improvement in the first few hours after the resuscitation seems to have the best prognostic value (15)

In patients with cardiac arrest the duration of brain ischemia has been shorter than in those with near drowning in whom many factors before the cardiac arrest (such as asphyxia changes in water electrolyte balance changes in blood pressure) can affect oxygen supply to the brain The time interval before effective resuscitation must also have been longer in near drowning

In connection with brain ischemia increasing intracranial pressure caused by brain swelling aggregation of blood particles and morphological changes can decrease the circulation (7) even with good lung function It is possible that both the transient and the progressive slowing in the EEGs obtained in our patients can be explained on this basis

Thus the brain lesions in near drowning can be compared to those of brain contusion where a worsening can happen 3–4 days after the accident corresponding to the development of brain oedema

Finally recent reports on the treatment of cerebral ischemia with high doses of barbiturates are most interesting In this treatment when the circulation is restored cerebral function is immediately depressed by giving large doses of thiobarbiturates Primate studies indicate a very marked improvement compared to previous results and there are also reports on the very favourable effects of this treatment on humans This should be held in mind when planning the future treatment of near drowning victims (2 17)

ACKNOWLEDGEMENT

This study has been supported by the Rinnekoti Foundation

REFERENCES

1 Bigelow W G Lindsay W K Harrison R C Gordon R A & Greenwood W F Oxygen transport and utilization in dogs at low body temperatures *Am J Physiol 160* 125 1950

2 Breivik H Feasibility of barbiturate loading of patients for brain resuscitation after cardiopulmonary arrest Abstracts of the *Second World Congress on Intensive Care* Paris 19–23 Sept 1977 p 165

3 Brendel W Kreislauf in Hypothermie *Verh Dtsch Ges Kreislaufforsch 23* 33 1957

4 Eriksson R Fredin H Gerdman P & Thorson J Sequelae of accidental near drowning in childhood *Scand J Soc Med 1* 3 1973

5 Fandel I & Bancalari E Near-drowning in children clinical aspects *Pediatrics 58* 573 1976

6 Goldberg S & Lewis M Play behaviour in the year old infant early sex differences *Child Dev 40* 21 1969

7 Hossmann K A & Zimmermann V Resuscitation of the monkey brain after 1 h complete ischemia I Physiological and morphological observations *Brain Res 81* 59 1974

8 Imbach P Kabus K & Tonz O Erfolgreiche Behandlung eines Ertrinkungsfalls nach 0 minuten Submersion *Schweiz Med Wochenschr 105* 1[illegible] 1975

9 King R B & Webster I W A case of recovery from drowning and prolonged anoxia *Med J Aust 1* 919 1964

10 Kvittingen T D & Naess A Recovery from drowning in fresh water *Br Med J 1* 1315 1963

11 Lougheed D W Janes J M & Hall G E Physiological studies in experimental asphyxia and drowning *Can Med Assoc J 40* 423 1939

12 Marcusson H Epidemiologie des Unfalls im Kindesalter *Z Gesamte Hyg 13* 222 1967

13 Modell J H *The pathophysiology and treatment of drowning and near drowning* Charles C Thomas Springfield Ill 1971

14 Modell J H Graves S A & Ketover A Clinical course of 91 consecutive near drowning victims *Chest 70* 231 1976

15 Pampiglione G & Harden A Resuscitation after cardiocirculatory arrest prognostic evaluation of early EEG findings *Lancet 1* 1261 1968

16 Pearn J Neurological and psychometric studies in children surviving freshwater immersion accidents *Lancet 1* 7 1977

17 Safar P & Nemoto E Brain resuscitation A summary of its present status *14th Congress of the Scandinavian Society of Anaesthesiologists* Uppsala Sweden June 28–July 2 1977

18 Siebke H Breivik H Rod T & Lind B Survival after 40 minutes submersion without cerebral sequelae *Lancet 1* 1275 1975

19 World Health Organization *The prevention of accidents in childhood* Report on a WHO seminar Copenhagen 1960

Submitted April 27 1978
Accepted Oct 13 1978

(S K) Research Department
Rinnekoti Institution
02980 ESPOO 98
Finland

Immunization Thirty one children had had one two or three injections of pertussis vaccine while 24 were not immunized Information was lacking in four cases The development of whooping attacks feeding difficulties or need for hospitalization could not be related to immunization status

Erythromycin treatment One child (six weeks[1]) had been given erythromycin prophylactically because of pertussis in the family Two days after having finished a ten day course he developed symptoms He was later hospitalized for five days because of feeding difficulties Three children were given erythromycin on the very first day of catarrhal symptoms One of them had a very mild disease with no whooping attacks The second child developed moderately severe whooping attacks while the third child developed typical whooping attacks with rather severe vomiting Ten children were given erythromycin on the second to fifth day of catarrhal symptoms Five of them had a mild disease without typical whooping attacks Twenty nine children were given erythromycin to reduce contagiousness after more than five days of symptoms usually when the whooping attacks had already started One of them had to be hospitalized because of diarrhoea

Laboratory data Blood leucocytes in 26 children (20 hospitalized) in the whooping stage ranged from 10 2–31 0 (mean 22) $\times 10^9$/l with 55 5 to 88 (mean 76) % mononuclear cells Seven children in the catarrhal stage with symptoms for less than one week had 7 8–11 8 (mean 9 6) $\times 10^9$ leucocytes/l with 33–51 (mean 43) % mononuclear cells

DISCUSSION

As only patients with positive cultures were included in this study the results might not give a completely true picture of pertussis in small children in this region In mild cases with no known exposure to pertussis the diagnosis might not be suspected while on the other hand in clinically typical cases culture might be considered unnecessary It is not likely though that the most serious cases have been missed as they should have appeared at one of the hospitals all of which have been interested in pertussis with a high frequency of cultures

It may therefore be concluded that pertussis in Sweden even in the youngest age group is in most cases a mild to moderately severe disease causing more social than medical complications Of the 59 patients only one with a congenital hypothyroidism was critically ill while as many as 19 never developed typical whooping attacks Secondary infections occurred in only five cases

There are many possible explanations for the declining severity of the disease Islur et al (3) and Miller & Fletcher (4) have shown that active immunization is of value but their results as well as the findings in this study show that even complete immunization does not always protect from typical whooping attacks Whether or not immunization with the Swedish vaccine predisposes to a milder course is not possible to evaluate from this uncontrolled material in which only six children were completely immunized Other possible reasons for the decreasing severity of the disease suggested by Islur et al (3) are antimicrobial therapy improved nursing care and supportive therapy and perhaps a decreased virulence of the causative organism

Antibiotics are certainly of value for the treatment of secondary bacterial complications Erythromycin treatment in the catarrhal stage might also affect the subsequent course of the disease as shown by Bass et al (2) The children treated early in this study had a somewhat milder course as six out of 13 did not develop whooping attacks compared to 13 out of 46 children not treated in the catarrhal stage but this difference was not statistically significant The fact that one child developed symptoms two days after having completed a ten day course of erythromycin given

[1] Age when symptoms started

Table 1 *Reasons for hospitalization in children with pertussis in different age groups*

Reasons for hospitalization	Age group			
	2–8 weeks	9–16 weeks	17–26 weeks	Total
Social reasons	3	6	4	13
Feeding difficulties and vomiting	2	2	1	5
Apnoea	0	2	1	3
Concurrent pneumonia	1	0	0	1
Fever	0	0	1	1
Diarrhoea (erythromycin induced)	0	0	1	1
Total number hospitalized	6	10	8	24
Non hospitalized children	11	9	15	35
Total	17	19	23	59

complicating obstructive bronchitis and had to be kept for observation for three weeks

The five children hospitalized because of feeding difficulties and serious vomiting were admitted in the second to fourth week of the disease Three of them had lost weight All improved satisfactorily and could be discharged after four to ten days in good condition

None of the other 15 hospitalized children had severe disease with apnoea secondary bacterial complications or serious feeding difficulties One of them was admitted because of diarrhoea caused by erythromycin given to reduce contagiousness Another was observed for two days because of fever which disappeared spontaneously Thus 13 children were hospitalized mainly for social reasons because the parents were worried or worn out after being kept awake by the nightly coughing attacks Most of these 13 children had moderately severe whooping attacks sometimes with cyanosis but were in good general condition and alert between the coughing attacks Two of them never developed typical whooping attacks at all One of the mothers of these children had pertussis herself and six parents had more than one child with the disease which explains why they could not take care of their children at home In three of these cases hospitalization lasted from two to four weeks owing to serious social problems (a pair of twins had a mother with a serious mental disorder and a girl had a father hospitaliz after having been assaulted)

Non hospitalized patients As many as of the 35 patients not requiring hospitaliz tion had a mild disease with no typical whoo ing attacks and little or no vomiting The re son why nasopharyngeal cultures were o tained was catarrhal symptoms or nonspeci cough in children who had been exposed pertussis in the family The second younge child (three weeks[1]) belonged to this grou The other 18 of the 35 non hospitalized ch dren had typical whooping attacks often wi short periods of cyanosis and vomiting b were in good general condition The younge child (two weeks[1]) belonged to this group

Secondary infections As already me tioned in the hospitalized group there w one case of bronchopneumonia and one ca of obstructive bronchitis A non hospitaliz child had a small bronchopneumonia (verifi by X ray) which was cured without antibiot treatment One child had an otitis and one h a tonsillitis (betahaemolytic streptococci cu tured)

Exposure to pertussis Thirty six of the children had caught the disease from old brothers or sisters Seven children had bee infected by cousins or children of neighbour In 16 cases the source of infection was n known

[1] Age when symptoms started

with whooping cough However this boy had a history of IRDS in the neonatal period

It is always hard to judge the severity of a disease in a retrospective study The absence of recorded complications does not necessarily mean that hospitalization was mainly for social reasons Only rarely in the notes of our 57 infants were social reasons mentioned as a contributory reason for admission Most infants required individual supervision by nurses able to give oxygen and use suction when necessary We share the opinion of other investigators that the relative lack of complications should be attributed to improved nursing care and not only to the fact that whooping cough may be milder

There seems to be general agreement that infants should be protected from whooping cough A general recommendation has been erythromycin for the index case to reduce its contagiousness and for the infant to possibly prevent the disease However we have noticed as has the author that in many infants (in his study 16/59 i e one third) the source of infection is not known The only means of protecting these infants is to reduce the incidence of the disease in older children by immunization This is why the Swedish Pediatric Association recommends that pertussis vaccine should still be used

Several antibiotics have been shown to be effective against *Bordetella pertussis* in vitro Unfortunately few clinical trials have been conducted to test its effect The drug of choice today erythromycin has been shown to rapidly eradicate the bacteria from the throat during treatment (2 3) However in comparison with drugs that were used earlier there are only a few patients that have been followed after discontinuation of erythromycin I think that many doctors today would welcome such a study in order to be able to better calculate the risk for exposed individuals

In two studies erythromycin was shown to be effective in preventing the spread of whooping cough among exposed babies in a nursery (1 4) This lead to its widespread use in Sweden In accordance with the findings of the authors of the study in Gothenburg we have also seen whooping cough in prophylactically treated infants Among 15 infants admitted to our hospital during 1978 three received this treatment at the same time as the index cases (siblings) They developed whooping cough in spite of this and had to be hospitalized for medical reasons

Margareta Eriksson

Dr Trollfors wants to make the following comments

After having reach the commens made by Dr Eriksson I would like to discuss some points Unfortunately Dr Eriksson misquoted me in writing The author concludes that whooping cough is a mild to moderately severe disease The correct citation should have been is *in most cases* a mild to moderately severe disease as I am aware that serious cases exist and also reported one in the study

I do not think that the material described by Dr Eriksson presents a worse picture of the disease than mine She found 56 hospitalised cases in 8 years whereas I found 24 hospitalised cases in one year even though the hospital in Stockholm received children for intensive care from a region twice as large as that of Gothenburg The main difference between our studies is that Dr Eriksson described only hospitalised cases while I tried to cover the whole spectrum of the disease including non hospitalised cases I feel that Dr Eriksson underestimates our colleagues on pediatric departments in writing The absence of recorded complications does not necessarily mean that hospitalization was mainly for social reasons Complications serious enough to motivate hospitalisation would surely have been noted in the records on admittance or discharge!

prophylactically is remarkable as Altmeier & Ayoub (1) have shown good effect of prophylactic erythromycin treatment

In the whooping stage erythromycin is only indicated to reduce *contagiousness or to treat* secondary bacterial complications It has probably been prescribed too widely as *most* of these children came from families in which other susceptible members already had the disease or must have been exposed before treatment started Almost all hospitalized children were treated with erythromycin even though they were kept separate from other children This might be justified if members of the staff are susceptible

The good nursing care at a modern paediatric hospital is naturally of value for children with serious whooping cough Most cases reported here though did not require hospitalization Many *of the other* infants stayed in hospital for only a few days and left with unchanged *symptoms so the period of hospital* stay could not possibly have affected the general course of the disease

Thus even in the youngest most susceptible age group there are many whooping cough patients with mild symptoms who have not been immunized not received early erythromycin treatment and only received basic nursing care from their parents at home This *leaves* room for speculation that the benign course of pertussis seen during the last two *decades is due to non medical factors such as* a generally improved resistance to infections related to better socio economic conditions and/or a decreased virulence of the causative organism

REFERENCES

1 Altemeier W A & Ayoub E M Erythromycin prophylaxis for pertussis *Pediatrics 59* 623 1977
2 Bass J W Klenk E L Kotheimer J B Linnemann C C & Smith M H D Antimicrobial treatment of pertussis *J Pediatr 75* 768 1969
3 Islur J Anglin C S & Middleton P J The whooping cough syndrome A continuing pediatric problem *Clin Pediatr 14* 171 1975
4 Linnemann *C C Ramundo* N Perlstein P H Minton S D Englender G S McCormick J B & Hayes P S Use of pertussis vaccine in an epidemic involving hospital staff *Lancet II* 540 1975
5 Melchior J C & Jessen O Hospitalsindtryk af kighosteepidemien 1973–1974 *Ugeskr Laeger 138* 1 8? 1976
6 Miller C L & Fletcher W B Severity of notified whooping cough *Br Med J I* 117 1976
7 Sarnaik A P Pensler L & Grege R H The use *of parenteral feeding in whooping cough Clin Ped 17* 409 1978

Submitted July 20 1978
Accepted Nov 9 1978

Department of Infectious Diseases
Östra sjukhuset
S-41685 Göteborg
Sweden

The Editor has asked Dr Margareta Eriksson Karolinska Institutet St Goran s Children s Hospital Stockholm Sweden to comment on the article on whooping cough

Whooping cough has achieved new and increasing interest during the last few years In most countries in the Western world it is still considered to be a serious disease in infants below the age of three to six months (3 5 6 7) The author of this retrospective survey concludes that whooping cough in Sweden is a mild to moderately severe disease As long as *there is no generally accepted definition* of what is a serious disease opinions will differ *between investigators* as *well* as with the group of infants studied

During the past eight years 57 infants less than six months of age (28 below 3 months) have been admitted to St Goran s Children s Hospital This hospital serves a population of 400 000 and in addition receives children for intensive care from an area with a *population* of 1 1/2 million Of these 57 infants three de*veloped such serious apneic spells* that artificial ventilation was required for a period of 4–21 days All of the infants were fullterm healthy babies of six to seven weeks of age In addition during this period artificial ventilation was also required for a 10 month old boy

Acta Pædiatr Scand 68 329–336 1979

A PROSPECTIVE STUDY ON THE INCIDENCE AND SIGNIFICANCE OF CONGENITAL CYTOMEGALOVIRUS INFECTION

H KERZEL ANDERSEN KARIN BROSTRØM[2] K BROGÅRD HANSEN[3] J LEERHØY M PEDERSEN[3] O ØSTERBALLE[4] U FELSAGER and S MOGENSEN

From the Institute of Medical Microbiology [2]Departments of Obstetrics and Gynaecology and [3]Paediatrics University of Aarhus [3]Departments of Paediatrics and [4]Obstetrics and Gynaecology County Hospital Viborg and the State Serum Institute Copenhagen Denmark

ABSTRACT Kerzel Andersen H Brostrøm Karin Brogård Hansen K Leerhøy J Pedersen M Østerballe O Felsager U and Mogensen S (Institute of Medical Microbiology Departments of Obstetrics and Gynaecology and Paediatrics University of Aarhus Departments of Paediatrics and Obstetrics and Gynaecology County Hospital Viborg and the State Serum Institute Copenhagen Denmark) A prospective study on the incidence and significance of congenital cytomegalovirus infection Acta Paediatr Scand 68 329 1979 — Screening of 3060 neonates for congenital cytomegalovirus (CMV) infection by virus excretion in the urine showed an overall incidence of 0 4% The incidence was about 1% for mothers between 16 and 25 years and only 0 2% for mothers between 25 and 35 No mothers over 35 years of age gave birth to congenitally infected infants The percentage of women in the child bearing age susceptible to CMV infection was estimated by the absence of CMV complement fixing antibodies in cord sera and ranged from 48% to 33% with increasing age None of the infected infants showed obvious signs of congenital CMV infection at birth At follow up two infants showed slight but transient symptoms compatible with a foetal infection a pair of premature twins exhibited retarded physical and psychomotor development but this could just as well be ascribed to the prematurity itself None of the infants had detectable CMV-IgM antibodies in cord sera but a trend towards elevated total IgM concentration in cord sera and elevated virus excretion titres appeared in the infants with symptoms With the very low incidence and no signs of sensomotor sequelae the preliminary conclusion is that foetal CMV infection in our population by no means has a significance to deserve routine screening or a vaccination programme

KEY WORDS Cytomegalovirus congenital infection

The classical cytomegalic inclusion disease of the newborn caused by cytomegalovirus (CMV) is well known The clinical picture exhibits a variety of symptoms the best known being those involving the brain and giving rise to microcephalus meningo-encephalitis and cerebral calcifications ocular lesions characterized by chorioretinitis and optic atrophy hepatosplenomegaly and haematological disturbances leading to trombocytopenic purpura and haemolytic anaemia

Since 1956 it has been possible to diagnose the infection *intra vitam* and 10 years later Hanshaw stated after an investigation of 17 cases that congenital infection was invariably associated with disease and a poor prognosis especially as regards future psychomotor development (9) However during recent years several investigations on the incidence and significance of congenital CMV infection have shown examples with slight and varied clinical manifestations as well as cases with no signs of overt disease at all (12 17 19 21 22) In these studies the symptomatology and incidence vary widely according to differences in the populations investigated and as to whether the diagnosis was established by virus isolation or antibody determinations (13 15 18) Therefore it seems important to perform a study of congenital CMV infection in a Scandinavian population especially as prophylaxis of the infection with a vaccine may become possible although the vaccine has not yet been sufficiently tested (6 16)

Details of "social reasons" were given in the article concerning 4 of 13 cases. As most of the others were hospitalised for less than five days it is unlikely that they were all seriously ill and still could leave the hospital after only a few days.

I agree with Dr Eriksson that good nursing care is of value in many cases, but however good nursing care may be at a modern hospital it could not possibly have influenced the course of the disease of the children who were never hospitalised (35 of 59), and again I want to stress that the majority of the hospitalised children left hospital after less than five days. I agree with Dr Eriksson that, if possible, small children should be protected fro[m] whooping cough, but erythromycin treatme[nt] should be used with some restriction as if to[o] widely used, resistant strains may appea[r]. Erythromycin can also cause gastrointestin[al] side effects and a child with whooping coug[h] *is not much helped by also having diarrhoe[a].*

Finally, I think the most important findi[ng] i[n] my study was that whooping cough even i[n] the smallest children can have a very variab[le] clinical course from serious cases needi[ng] assisted ventilation to very mild cases with nonspecific cough and good general condition.

B Trollfors

Table 1 *The incidence of congenital CMV infection in different age groups of mothers*

Age group (years)	Study groups: University Hospital No	%	County Hospital No	%	Total No	Mothers with infected infant: No	Incidence at age group (%)
16–19	51	? 5	48	4 6	99	1	1 0
0– 4	418	0 7	30_	?8 9	7 0	7	1 0
?5– 9	830	41	4?5	40 8	1 ?55	?	0 ?
30–34	499	?4 7	183	17 6	682	1	0 2
35–40	174	8 6	76	7 3	?50	0	0
≥40	45		9	0 9	54	0	0
Total	_ 017		1 043		3 060	11	0 4

One mother gave birth to a pair of infected twins

haemolysis trombocytopenia and hepatobiliary involvement *but only infected infants were subjected to opthalmological* examination and X ray of the skull chest and extremities

RESULTS

The incidence of congenital CMV infection
A total number of 478 pools of urines from the neonates of 2 017 deliveries (30 pairs of twins) at the University Hospital and 366 pools from 1 043 deliveries (4 pairs of twins) at the County Hospital were cultured for CMV A total of 10 pools seven from the University Hospital and 3 from the County Hospital yielded CMV whereas no other viruses were detected By examination of the individually stored urines the excreting infants were easily found in all cases except one In this case the excreting infant was found after reisolation attempts from the respective infants at 7 weeks of age *In the 8 pools of CMV containing urines* only one infant was found to be the excreter whereas in two pools two unrelated infants and two twins respectively were found to be infected By using this method the diagnosis was established between 14 and 26 days after the arrival of the specimen except in the case in which new specimens had to be collected

Thus 8 infants from 7 deliveries (0 4%) at the University Hospital and 4 infants from the County Hospital (0 4%) were found to be infected at birth The incidence in relation to the age of the mothers is shown in Table 1 It is relatively high (1%) in the age groups under 25 years and much lower (0 2%) in the age groups between 25 and 35 This difference is statistically significant ($P<0.01$) None of the mothers over 35 years delivered congenitally infected infants No difference concern

Table 2 *Number of CMV seropositive individuals in different age groups*

Age groups (years)	Female blood donors No	%	Mothers[b] from University Hospital No	%	County Hospital No	%
16– 4	40 66	61	35/66	53	51/99	5?
5–?9	51/90	57	77/137	56	68/1?8	53
30–34	6/39	67	4 6?	67	35/60	58
35–39	13/17	76	16/ 5	64	1 /18	67
40–44	13/17	76	5/6		1/	
Total	143/? 9	6	175/ 96	59	167/307	54

H pital staff
The serological finding in mothers are based on studies of cord sera from their infants

For this purpose 3000 infants born in two Danish hospitals were screened for urinary CMV excretion at birth and infected infants were followed up to an age ranging from 8 months to 2 years

MATERIALS AND METHODS

Study groups The study included infants born at the County Hospital of Viborg a general hospital which accepts all deliveries in that county and infants born at the Department of Obstetrics and Gynaecology University Hospital Aarhus The University Hospital receives mostly women with pathological and potentially pathological pregnancies and deliveries

The study from the County Hospital covered the period from November 1974 through October 1975 and 1047 of 1109 neonates (94%) were tested The study from the University Hospital ran from March 1976 through February 1977 and included 2047 of 2460 infants (83%) It was planned to collect urine from all neonates during their one week stay in the hospital but isolation attempts had to be stopped during Easter and Christmas Therefore infants born during these periods were excluded and so were some other infants because of unsuccessful collection or urine Isolation attempts from 67 urines failed in the laboratory because of broken glasses or contamination of the cultures with bacteria or fungi

Furthermore material from 36 stillborn or perinatally dead infants were included in the study This constitutes about one half of the total number of cases the other half being excluded because of postmortal autolysis

Specimens Urine from infants was collected in plastic urinary bags during the first 5 days of life and kept at 4–10°C until transport to the laboratory From the County Hospital the specimens were posted overnight in containers at 0–10°C (Nunc) whereas the material from the University Hospital arrived in the laboratory within one hour The hospitals were instructed never to freeze the specimens The dead infants were autopsied and small specimens of the lung liver and kidney were cultured for virus When possible blood from the umbilical cord was drawn for serum antibody study

Virus isolation procedure Monolayers of human embryonic lung fibroblasts in 200 ml bottles were used for virus isolation as described previously (2) Urine was mixed with equal parts of Eagle minimum essential medium (MEM) with 10% calf serum and adjusted to pH 7 2 with a 2 8% solution of sodium bicarbonate and stored in that condition for one month at 4 C For the screening procedure 1 ml of pH adjusted urines from 2–5 infants were pooled and added to a tissue culture for 1 hour at 37 C with tilting of the bottle every 15 min After the adsorption the cultures were overlaid with MEM containing 2% calf serum and antibiotics (gentamycin and mycostatin) for 2 days after which the medium was changed to Dulbecco s special medium with 2% serum and antibiotics Cultures were observed during the first few days for toxic effect of the urines and if necessary the medium was changed or the amount of serum was increased for a few days to keep the cells in good condition The medium was changed once a week and the cultures were observed for cytopathic effect twice a week for a total of 4 weeks Cultures which were negative for virus growth after more than 20 days of culture but were lost during the fourth week were considered negative and included in the material Individual cultures for virus were made of stored urines from pools yielding growth of CMV Later samples from virus excreters were transported in 2 ml of MEM + 10% calf serum Autopsy tissue was squeezed and added to a culture for 3 hours after which the culture medium was changed

Viruses were identified as CMV by their slowly developing cytopathic effect in tissue culture and the formation of small plaques stainable with methylene blue The number of plaques were counted before secondary plaques had formed and on the basis of this the virus excretion titre was estimated

Check of the isolation procedure The transport system and the sensitivity of the culture procedure were controlled 25 times during the study Urine specimens from 6 known CMV-excreting infants were posted overnight like the urine specimens from the County Hospital and cultured for virus As CMV was isolated in each instance the procedure was regarded as sufficient

Serological studies The Ad 169 strain was employed as the source of antigen for all serological procedures Complement fixing (CF) antibodies were determined by a micromethod as described earlier (2)

Levels of IgM in cord sera were measured by immunodiffusion on plates from Behringwerke AG Germany All serum samples from congenitally infected infants and controls were investigated simultaneously and levels at <18 mg/100 ml were considered as normal

Studies of specific CMV IgM antibodies using an immunofluorescence test were done as previously described (3) The sera were tested in dilutions from 1 10 with known positive and negative controls

Clinical assessment Most of the infected infants from the University Hospital were born after a more or less complicated pregnancy and/or delivery A control group was selected from the same department The next two infants of the same sex and birth weight ±100 g and with the mothers age matched were selected Examination took place at 8 weeks and 8 months of age No controls were available for the pair of infected premature twins Infected infants from the County Hospital the mothers of whom comprised a general delivery population were evaluated without controls as soon as the diagnosis was established and every third month thereafter until the age of 2 years

Records of the pregnancy delivery and neonatal period were scrutinized retrospectively with respect to risk factors as maternal abnormalities and pregnancy and birth complications and clinical signs of CMV infection

The clinical assessment consisted of a complete physical and neurological examination The infants were not exposed to a refined infant intelligence test but milestones for gross sensomotor development were used in the judgement of mental and motor capabilities

At the first visit a complete blood count and blood chemistry determination were performed for signs of

Table 1 *The incidence of congenital CMV infection in different age groups of mothers*

Age group (years)	Study groups: University Hospital No	%	County Hospital No	%	Total No	Mothers with infected infant: No	Incidence at age group (%)
16–19	51	2 5	48	4 6	99	1	1 0
20–24	418	20 7	30[illegible]	8 9	720	7	1 0
25–29	830	41 [illegible]	475	40 8	1 [illegible]55	2	0 2
30–34	499	24 7	183	17 6	68[illegible]	1	0 2
35–40	174	8 6	76	7 3	50	0	0
≥40	45	2 2	9	0 9	54	0	0
Total	2 017		1 043		3 060	11	0 4

One mother gave birth to a pair of infected twins

haemolysis trombocytopenia and hepatobiliary involvement but only infected infants were subjected to opthalmological examination and X ray of the skull chest and extremities

RESULTS

The incidence of congenital CMV infection
A total number of 478 pools of urines from the neonates of 2 017 deliveries (30 pairs of twins) at the University Hospital and 366 pools from 1 043 deliveries (4 pairs of twins) at the County Hospital were cultured for CMV A total of 10 pools seven from the University Hospital and 3 from the County Hospital yielded CMV whereas no other viruses were detected By examination of the individually stored urines the excreting infants were easily found in all cases except one In this case the excreting infant was found after reisolation attempts from the respective infants at 7 weeks of age In the 8 pools of CMV containing urines only one infant was found to be the excreter whereas in two pools two unrelated infants and two twins respectively were found to be infected By using this method the diagnosis was established between 14 and 26 days after the arrival of the specimen except in the case in which new specimens had to be collected

Thus 8 infants from 7 deliveries (0 4%) at the University Hospital and 4 infants from the County Hospital (0 4%) were found to be infected at birth The incidence in relation to the age of the mothers is shown in Table 1 It is relatively high (1%) in the age groups under 25 years and much lower (0 2%) in the age groups between 25 and 35 This difference is statistically significant ($P<0\ 01$) None of the mothers over 35 years delivered congenitally infected infants No difference concern

Table 2 *Number of CMV seropositive individuals in different age groups*

Age groups (years)	Female blood donors* No	%	Mothers† from University Hospital No	%	County Hospital No	%
16– 4	40 66	61	35/66	53	51/99	5[illegible]
5–29	51/90	57	77/137	56	68/1 8	53
30–34	6/39	67	4 /6	67	35/60	58
35–39	13/17	76	16/ 5	64	1 /18	67
40–44	13/17	76	5 6		1/2	
Total	143/[illegible] 9	6	175/ 96	59	167/307	54

*Hospital staff
†The serological findings in mothers are based on studies of cord sera from their infants

Table 3 *The main features of the 12 infected infants*

Case No	Birth weight and length (g/cm)	Clinical data: Neonatal period	Clinical data: At follow up	Virus excretion titre (×10³ PFU/ml): 1 week	2 months	8 months
1	3 650/50	Jaundice	Asymptomatic	2 5	2 5	0 1
2	4 000/53	Haemangiomata	Asymptomatic	2 0	4 0	0 05
3	3 180/51	Jaundice IgM 27 mg/100 ml	Non specific osseous changes reticulocytosis		12 5	70
4	3 700/52	Normal IgM 100 mg/100 ml	Liver and spleen enlargement specific osseous changes reticulocytosis	15		12 5
5	3 560/50	Haemangiomata	Head circumference +2 S D to length[b]	15	5	2 5
6	2 920/50	Small for date	Specific osseous changes	15	6	2 0
7	1 000/37	Gemellus jaundice IgM 30 mg/100 ml	Retarded psychomotor development mild spastic diplegia failure to thrive deficient rearing[a]	≥100		≥100
8	1 380/41	Gemellus jaundice IgM 60 mg/100 ml	Retarded psychomotor development failure to thrive deficient rearing[b]	≥100		12 5
9	2 800/49	Small for date jittering and hyperexitability	Asymptomatic			
10	2 450/49	Small for date	Asymptomatic			
11	4 200/55	Normal	Asymptomatic			
12	3 400/51	Normal	Asymptomatic			

[a] Normalized at 8 months
[b] Unchanged at 8 months

ing the incidence in relation to age groups was found between the two study groups neither was any seasonal variation noticed All mothers except one were primipara Virus was not isolated from any of the dead infants CMV excretion was continued in all infected infants Table 3 shows the virus excretion titres of the infected infants from the University Hospital The first two cases were characterized by fairly low and decreasing titres whereas the next four showed about 10 times higher levels of CMV uria The twins excreted about 10⁴ plaque forming units/ml and this high level persisted in one whereas a 10 fold decrease was seen in the other during the follow up Throat swabs from the congenitally infected infants were positive in all but two cases

Serological studies Since CMV antibodie are transferred transplacentally the degree c previous exposure of the mothers to the viru was evaluated by examining about 300 ran domly taken cord sera from each hospita group for CF antibodies to CMV The result were compared with the degree of seroposi tivity found in a group of female blood donor from a hospital staff As seen from Table the mean rate of seropositivity varies betwee 54 and 62% and a typical rise in seropositivit is seen with increasing age in all three group

Cord sera from 11 of the infected infan and from the 12 control infants were avai able for determination of IgM concentratio All control infants and all but 4 of the infecte infants showed levels of IgM less than 18 m 100 ml The infected infants with elevated co

centration of IgM were cases Nos 3 and 4 and the pair of twins (Table 3) It was however not possible to demonstrate specific CMV IgM antibodies in any of these sera

Clinical findings Table 3 summarizes the main features of the 12 infected infants in the study Evaluation of the six of the eight infected infants from the University Hospital (cases 1–6) and of the 12 controls showed that risk factors in respect to pregnancy and delivery were comparable None of the infected infants showed obvious symptoms suggestive of CMV infection Mean Apgar scores at 1 min were 9 5 and 9 2 respectively with the highest score in the infected group Two of the infected infants were jaundiced while only one of the controls exhibited significant hyperbilirubinaemia No infants in either group showed major congenital defects but two infected infants were born with small haemangiomata of the strawberry type Both infected and control infants were discharged after a mean period of 6 3 days but mean weight losses from birth to the fifth day of life was greater in the infected group (mean 183 g S D 116 g *vs* 96 g S D 180 g $P=0\ 15$)

At 8 weeks and 8 months of age all infants were in good health but physical examination at the first visit revealed in one viruric infant enlargement of the liver and spleen which were 2 cm below the costal margins The two haemangiomata were regressing normally Growth and nutritional status including weights of all infants were within normal limits The head circumference of one viruric infant differed −2 S D to length on both visits though she had no microcephalic look The head circumference of all other infants were appropriate to length centiles No neurological defects or hearing impairments were found and the psychomotor development at 8 month of age was within normal limits in all cases

The two remaining patients from the University Hospital fraternal twins (cases 7–8) were born of a 22 year-old Turkish mother They were nursed in incubators for one month but only one week of oxygen supply was necessary Except for slight hyperbilirubinaemia they behaved well and there were no specific signs of systemic CMV infection Discharge took place to an infant home because of bad parental and social circumstances but from 9 months of age they were reared at home Their physical and psychomotor development was moderately retarded at follow up at 5 and 11 months of age

Of the 4 infants from the County Hospital (cases 9–12) two were born small for gestational ages according to standard criteria The neonatal period gave no suspicion of CMV infection One of the infants born small for gestational age was born after vacuum extraction because of hypertension *sub partu* This infant was jittering and showed hyperexcitability in the first few weeks the others behaved normally All 4 infants followed to the age of 2 years were normal as regard physical examination psychomotor development and hearing

Laboratory investigations as measured at the first examination None of the infants had trombocytopenia There were signs of overt haemolysis measured by haemoglobin MCV reticulocytes and serum bilirubin although two viruric infants exhibited the highest reticulocyte counts of 4 8% and 4 2% (infected mean 2 3% S D 2 0% and control mean 1 5% S D 1 0% not significant) Mean total lymphocyte counts determined from the total white blood count multiplied by the per cent of lymphocytes on differential white cell count were significantly higher in the infected group (mean $9\ 56\times10^{9}/l$ S D $2\ 00\times10^{9}/l$ and $6\ 54\times10^{9}/l$ S D $2\ 50\times10^{9}/l$ $P=0\ 01$) No hepatobiliary involvement was present as judged by serum GOT and alkaline phosphatase Urine analyses for albumin reducing substances and pyuria were unremarkable

Opthalmological and X ray examinations Opthalmological examination in the 12 infected infants revealed no visual disturbances microphthalmos or chorioretinitis X ray examination of the skull showed no cerebral cal

Table 3 *The main features of the 12 infected infants*

Case No	Birth weight and length (g/cm)	Clinical data: Neonatal period	Clinical data: At follow up	Virus excretion titre (× 10² PFU/ml): 1 week	2 months	8 months
1	3 650/50	Jaundice	Asymptomatic	2 5	2 5	0 1
2	4 000/53	Haemangiomata	Asymptomatic	2 0	4 0	0 05
3	3 180/51	Jaundice IgM 27 mg/100 ml	Non specific osseous changes reticulocytosis		12 5	20
4	3 700/52	Normal IgM 100 mg/100 ml	Liver and spleen enlargement specific osseous changes reticulocytosis	15		12 5
5	3 560/50	Haemangiomata	Head circumference +2 S D to length[a]	15	5	2 5
6	2 920/50	Small for date	Specific osseous changes	15	6	2 0
7	1 000/37	Gemellus jaundice IgM 30 mg/100 ml	Retarded psychomotor development mild spastic diplegia failure to thrive deficient rearing[b]	≥100		≥100
8	1 380/41	Gemellus jaundice IgM 60 mg/100 ml	Retarded psychomotor development failure to thrive deficient rearing[b]	≥100		12 5
9	2 800/49	Small for date jittering and hyperexitability	Asymptomatic			
10	2 450/49	Small for date	Asymptomatic			
11	4 200/55	Normal	Asymptomatic			
12	3 400/51	Normal	Asymptomatic			

[a] Normalized at 8 months
[b] Unchanged at 8 months

ing the incidence in relation to age groups was found between the two study groups neither was any seasonal variation noticed All mothers except one were primipara Virus was not isolated from any of the dead infants CMV excretion was continued in all infected infants Table 3 shows the virus excretion titres of the infected infants from the University Hospital The first two cases were characterized by fairly low and decreasing titres whereas the next four showed about 10 times higher levels of CMV uria The twins excreted about 10⁴ plaque forming units/ml and this high level persisted in one whereas a 10 fold decrease was seen in the other during the follow up Throat swabs from the congenitally infected infants were positive in all but two cases

Serological studies Since CMV antibodie are transferred transplacentally the degree o previous exposure of the mothers to the viru was evaluated by examining about 300 ran domly taken cord sera from each hospita group for CF antibodies to CMV The result were compared with the degree of seroposi tivity found in a group of female blood donor from a hospital staff As seen from Table the mean rate of seropositivity varies betweer 54 and 62% and a typical rise in seropositivit is seen with increasing age in all three groups

Cord sera from 11 of the infected infants and from the 12 control infants were avail able for determination of IgM concentration All control infants and all but 4 of the infected infants showed levels of IgM less than 18 mg/ 100 ml The infected infants with elevated con

These latter diseases were not probable in our cases as the infants were seronegative for IgM rubella antibodies and the mothers were WR negative The prognostic value of deter mining virus excretion titres is uncertain There are differences between the infants both in the initial titres and the titres at follow up However later examination of the chil dren is needed to draw any conclusions as to the significance of this parameter Melish & Hanshaw (13) found a correlation between the presence of specific CMV IgM antibodies and severe systemic damage Four of our in fants had elevated levels of IgM antibodies but none had specific CMV IgM antibodies at birth

Foetal infection with CMV may occur both during a primary infection of the mother and in mothers who are immune during the whole pregnancy (19) The reason why some infants sustain severe damage while the majority of the infants are asymptomatic is unknown but it is most likely as in congenital rubella that a primary infection early in preg nancy may be most dangerous to the foetus (1 14) On the other hand examples of asympto-matic congenital infections in infants whose mothers had a serologically proven primary infection during pregnancy are known (19) On the basis of studies in siblings consecu tively infected in utero (7 11 19 23) and studies in mothers who were immune precon ceptionally (19) it seems justified to conclude that congenital infection in infants born of immune mothers are asymptomatic It is un known whether the transmission in both primary and reactivated infections occurs transplacentally during a viraemia or the foetus may be infected via an ascending infec tion from the cervix which is known to be a site of predilection for CMV during preg nancy (17)

A continued follow up of the present series will be performed but may not provide further information of the significance of congenital CMV infection with the strong impact environ ment has with increasing age on infant intel ligence and performance Starr et al reported that infants who were asymptomatic at birth and at one year of age did not either show any sensoneural impairment at four years (21) Our preliminary conclusion therefore is that the foetal CMV infection observed in our limited series is not of sufficient signifi cance to deserve routine screening for CMV infection or a vaccination programme in our population

ACKNOWLEDGEMENTS

The authors wish to thank Johannes Ipsen D M Sc M P H Professor of Epidemiology and Medical Statis tics University of Aarhus for valuable assistance in the planing of this study This work was supported by Statens Lægevidenskabelige Forskningsråd Grant No 51 5704

REFERENCES

1 Altschuler G & McAdams A J Cytomegalic inclusion disease of a nineteen week fetus *Am J Obstet Gynecol* 111 295 1971
Andersen H K & Spencer E S Cytomegalo-virus infection among renal allograft recipients *Acta Med Scand* 186 7 1969
3 Andersen P & Andersen H K Immunoglobulin levels and specific viral antibodies in relation to smooth muscle antibodies in cytomegalovirus in fection *J Clin Lab Immunol* 1979 in press
4 Berenberg W & Nankervis G Long term follow up of cytomegalic inclusion disease of infancy *Pediatrics* 46 403 1970
5 Collaborative study Cytomegalovirus infection in the North West of England *Arch Dis Child* 45 513 1970
6 Elek S D & Stern H Development of a vaccine against mental retardation caused by cytomegalovirus infection in utero *Lancet* I 1 1974
7 Embil J A Ozere R L & Haldane E V Congenital cytomegalovirus infection in two siblings from consecutive pregnancies *J Ped tr* 77 417 1970
8 Graham C B Thal A & Wassum C S Rubella like bone changes in congenital cytomegalic inclusion disease *Radiology* 94 39 1970
9 Hanshaw J B Congenital and aquired cytomegalo-virus infection *Pediatr Clin North Am* 13 279 1966
10 Krech U Complement fixing antibodies against cytomegalovirus in different parts of the world *Bull Wld Hlth O g* 49 103 1973
11 Krech U Konjajev Z & Jung M Congenital cytomegalovirus infection in siblings from consecu tive prgnancies *Helv Paediat Acta* 26 355 1971
12 Kumar M L Nankervis G A & Gold E Inap-parent congenital cytomegalovirus infection *N Engl J Med* 288 1370 1973

cifications in any of the infants and chest X rays give no evidence of cardial or pulmonary disease In two children (cases 4 and 6) X ray of the lower extremities showed pronounced osseous changes consisting of translucent spots interspersed between bands of sclerosis in and near the metaphyses In another (case 3) periostal coating of the long bone diaphyses was seen The lesions had healed at 8 months but with marked periostal reactions left in all three infants

DISCUSSION

With the diagnosis of congenital CMV infection documented by the presence of CMV in the urine before the age of 5 days we found an incidence of 0 4% in our population This incidence is lower than in any other studies based on the same diagnostic procedure (12 19 21) Reported incidences of 1–3 5% are from previous studies predominantly composed of young mothers and with the highest rate in neonates whose mothers are both young and recruited from low socio economic populations The mean age of the parturient women in our study population was higher than in these studies and our affluent society will further reduce the low risk of congenital CMV infection in our child bearing women The general prevalence of CMV infection in fertile women was estimated on the basis of the presence of CF antibodies in cord sera as blood specimens from mothers were not available Our figures for seropositivity correspond well with those of other studies in populations comparable with ours (10) showing a high but decreasing percentage of susceptible women in the child bearing age Parallel to this a decrease in the number of congenitally infected infants was seen

The virus isolation procedure in which urines are pooled and cultured individually if necessary has been used with success in previous studies (20) In our hands this resource saving procedure also functioned satisfactorily To increase the sensitivity of the isolation procedure we used monolayers in bottles of 50 cm² and it is our impression that the isolates were given the necessary growth conditions Thus our low incidence seems to be real and not caused by a deficient isolation procedure

The clinical findings in the infected infants in the present study agree with previous reports (4 5 13 15 20) and confirm the inapparent or mild manifestations of most congenital CMV infections found by screening Common to these investigations are that few or none of the infants had clinically recognizable illness in the immediate postnatal period specifically no symptoms from the brain or eyes which carry a poor prognosis for future psychomotor development Hanshaw has estimated the frequency of this discouraging form to be about 10% of infected neonates (13)

Follow up of the present infected children showed that the child with slight cerebral symptoms perinatally was doing well later on and the complicated delivery rather than CMV could be responsible for the symptoms

Series in which a high rate of unfavourable clinical outcome have been reported although the infants were asymptomatic at birth consisted of selected populations of young mothers from low socio economic classes giving birth to a high percentage of premature infants (13 21) In many of these infants it is not possible to make any pathogenetic conclusions It can well be that the infection causes the prematurity or that the infection alone can be responsible for the sequelae observed In our infected premature twins without any objective signs of systemic CMV infection we are inclined to ascribe their retarded physical and psychomotor development to prematurity slight neonatal asphyxia and bad rearing

The diagnostic value of X ray examination of the long metaphyseal zones of the long bones is noteworthy The osseous changes first described by Graham et al (8) are not specific for CMV but are seen in other intrauterine infections such as rubella and syphilis

These latter diseases were not probable in our cases as the infants were seronegative for IgM rubella antibodies and the mothers were WR negative. The prognostic value of determining virus excretion titres is uncertain. There are differences between the infants both in the initial titres and the titres at follow up. However later examination of the children is needed to draw any conclusions as to the significance of this parameter. Melish & Hanshaw (13) found a correlation between the presence of specific CMV IgM antibodies and severe systemic damage. Four of our infants had elevated levels of IgM antibodies but none had specific CMV IgM antibodies at birth.

Foetal infection with CMV may occur both during a primary infection of the mother and in mothers who are immune during the whole pregnancy (19). The reason why some infants sustain severe damage while the majority of the infants are asymptomatic is unknown, but it is most likely, as in congenital *rubella*, that a primary infection early in pregnancy may be most dangerous to the foetus (1, 14). On the other hand, examples of asymptomatic congenital infections in infants whose mothers had a serologically proven primary infection during pregnancy are known (19). On the basis of studies in siblings consecutively infected in utero (7, 11, 19, 23) and studies in mothers who were immune preconceptionally (19), it seems justified to conclude that congenital infection in infants born of immune mothers are asymptomatic. It is unknown whether the transmission in both primary and reactivated *infections* occurs transplacentally during a viraemia, or the foetus may be infected via an ascending infection from the cervix, which is known to be a site of predilection for CMV during pregnancy (17).

A continued follow up of the present series will be performed but may not provide further information of the significance of congenital CMV infection with the strong impact environment has with increasing age on infant intelligence and performance. Starr et al. reported that infants who were asymptomatic at birth and at one year of age did not either show any sensoneural impairment at four years (21). Our preliminary conclusion therefore is that the foetal CMV infection observed in our limited series is not of sufficient significance to deserve routine screening for CMV infection or a vaccination programme in our population.

ACKNOWLEDGEMENTS

The authors wish to thank Johannes Ipsen, D.M.Sc., M.P.H., Professor of Epidemiology and Medical Statistics, University of Aarhus, for valuable assistance in the planing of this study. This work was supported by Statens Lægevidenskabelige Forskningsråd, Grant No. 512-5704.

REFERENCES

1. Altschuler, G. & McAdams, A. J.: Cytomegalic inclusion disease of a nineteen week fetus. *Am J Obstet Gynecol 111*: 295, 1971.
2. *Andersen, H. K. & Spencer, E. S.*: Cytomegalovirus infection among renal allograft recipients. *Acta Med Scand 186*: 7, 1969.
3. Andersen, P. & Andersen, H. K.: Immunoglobulin levels and specific viral antibodies in relation to smooth muscle antibodies in *cytomegalovirus* infection. *J Clin Lab Immunol* 1979, in press.
4. Berenberg, W. & Nankervis, G.: Long term follow up of cytomegalic inclusion disease of infancy. *Pediatrics 46*: 403, 1970.
5. Collaborative study: Cytomegalovirus infection in the North West of England. *Arch Dis Child 45*: 513, 1970.
6. Elek, S. D. & Stern, H.: Development of a vaccine against mental retardation caused by cytomegalovirus infection in utero. *Lancet I*: 1, 1974.
7. Embil, J. A., Ozere, R. L. & Haldane, E. V.: Congenital cytomegalovirus infection in two siblings from consecutive pregnancies. *J Pediatr 77*: 417, 1970.
8. Graham, C. B., Thal, A. & Wassum, C. S.: Rubella-like bone changes in congenital cytomegalic inclusion disease. *Radiology 94*: 39, 1970.
9. Hanshaw, J. B.: Congenital and aquired cytomegalovirus infection. *Pediatr Clin North Am 13*: 279, 1966.
10. Krech, U.: Complement fixing antibodies against cytomegalovirus in different parts of the world. *Bull Wld Hlth Org 49*: 103, 1973.
11. Krech, U., Konjajev, Z. & Jung, M.: Congenital cytomegalovirus infection in siblings from consecutive pregnancies. *Helv Paed atr Acta 26*: 355, 1971.
12. Kumar, M. L., Nankervis, G. A. & Gold, E.: Inapparent congenital cytomegalovirus infection. *N Engl J Med 288*: 1370, 1973.

cifications in any of the infants and chest X rays gave no evidence of cardial or pulmonary disease In two children (cases 4 and 6) X ray of the lower extremities showed pronounced osseous changes consisting of translucent spots interspersed between bands of sclerosis in and near the metaphyses In another (case 3) periostal coating of the long bone diaphyses was seen The lesions had healed at 8 months but with marked periostal reactions left in all three infants

DISCUSSION

With the diagnosis of congenital CMV infection documented by the presence of CMV in the urine before the age of 5 days we found an incidence of 0 4% in our population This incidence is lower than in any other studies based on the same diagnostic procedure (12 19 21) Reported incidences of 1–3 5% are from previous studies predominantly composed of young mothers and with the highest rate in neonates whose mothers are both young and recruited from low socio-economic populations The mean age of the parturient women in our study population was higher than in these studies and our affluent society will further reduce the low risk of congenital CMV infection in our child bearing women The general prevalence of CMV infection in fertile women was estimated on the basis of the presence of CF antibodies in cord sera as blood specimens from mothers were not available Our figures for seropositivity correspond well with those of other studies in populations comparable with ours (10) showing a high but decreasing percentage of susceptible women in the child bearing age Parallel to this a decrease in the number of congenitally infected infants was seen

The virus isolation procedure in which urines are pooled and cultured individually if necessary has been used with success in previous studies (20) In our hands this resource saving procedure also functioned satisfactorily To increase the sensitivity of the isolation procedure we used monolayers in bottles of 50 cm² and it is our impression that the isolates were given the necessary growth conditions Thus our low incidence seems to be real and not caused by a deficient isolation procedure

The clinical findings in the infected infants in the present study agree with previous reports (4 5 13 15 20) and confirm the inapparent or mild manifestations of most congenital CMV infections found by screening Common to these investigations are that few or none of the infants had clinically recognizable illness in the immediate postnatal period specifically no symptoms from the brain or eyes which carry a poor prognosis for future psychomotor development Hanshaw has estimated the frequency of this discouraging form to be about 10% of infected neonates (13)

Follow up of the present infected children showed that the child with slight cerebral symptoms perinatally was doing well later on and the complicated delivery rather than CMV could be responsible for the symptoms

Series in which a high rate of unfavourable clinical outcome have been reported although the infants were asymptomatic at birth consisted of selected populations of young mothers from low socio-economic classes giving birth to a high percentage of premature infants (13 21) In many of these infants it is not possible to make any pathogenetic conclusions It can well be that the infection causes the prematurity or that the infection alone can be responsible for the sequelae observed In our infected premature twins without any objective signs of systemic CMV infection we are inclined to ascribe their retarded physical and psychomotor development to prematurity slight neonatal asphyxia and bad rearing

The diagnostic value of X ray examination of the long metaphyseal zones of the long bones is noteworthy The osseous changes first described by Graham et al (8) are not specific for CMV but are seen in other intrauterine infections such as rubella and syphilis

These latter diseases were not probable in our cases as the infants were seronegative for IgM rubella antibodies and the mothers were WR negative The prognostic value of deter mining virus excretion titres is uncertain There are differences between the infants both in the initial titres and the titres at follow up However later examination of the chil dren is needed to draw any conclusions as to the significance of this parameter Melish & Hanshaw (13) found a correlation between the presence of specific CMV IgM antibodies and severe systemic damage Four of our in fants had elevated levels of IgM antibodies but none had specific CMV IgM antibodies at birth

Foetal infection with CMV may occur both during a primary infection of the mother and in mothers who are immune during the whole pregnancy (19) The reason why some infants sustain severe damage while the majority of the infants are asymptomatic is unknown but it is most likely as in congenital rubella that a primary infection early in preg nancy may be most dangerous to the foetus (1 14) On the other hand examples of asympto matic congenital infections in infants whose mothers had a serologically proven primary infection during pregnancy are known (19) On the basis of studies in siblings consecu tively infected in utero (7 11 19 23) and studies in mothers who were immune precon ceptionally (19) it seems justified to conclude that congenital infection in infants born of immune mothers are asymptomatic It is un known whether the transmission in both primary and reactivated infections occurs transplacentally during a viraemia or the foetus may be infected via an ascending infec tion from the cervix which is known to be a site of predilection for CMV during preg nancy (17)

A continued follow up of the present series will be performed but may not provide further information of the significance of congenital CMV infection with the strong impact environ ment has with increasing age on infant intel ligence and performance Starr et al reported that infants who were asymptomatic at birth and at one year of age did not either show any sensoneural impairment at four years (21) Our preliminary conclusion therefore is that the foetal CMV infection observed in our limited series is not of sufficient signifi cance to deserve routine screening for CMV infection or a vaccination programme in our population

ACKNOWLEDGEMENTS

The authors wish to thank Johannes Ipsen D M Sc M P H Professor of Epidemiology and Medical Statis tics University of Aarhus for valuable assistance in the planing of this study This work was supported by Statens Lægevidenskabelige Forskningsråd Grant No 51? 5704

REFERENCES

1 Altschuler G & McAdams A J Cytomegalic inclusion disease of a nineteen week fetus *Am J Obstet Gynecol 111* ?95 1971
2 Andersen H K & Spencer E S Cytomegalo-virus infection among renal allograft recipients *Acta Med Scand 186* 7 1969
3 Andersen P & Andersen H K Immunoglobulin levels and specific viral antibodies in relation to smooth muscle antibodies in cytomegalovirus in fection *J Clin Lab Immunol* 1979 in press
4 Berenberg W & Nankervis G Long term follow up of cytomegalic inclusion disease of infancy *Pediatrics 46* 403 1970
5 Collaborative study Cytomegalovirus infection in the North West of England *Arch Dis Child 45* 513 1970
6 Elek S D & Stern H Development of a vaccine against mental retardation caused by cytomegalovirus infection in utero *Lancet I* 1 1974
7 Embil J A Ozere R L & Haldane E V Congenital cytomegalovirus infection in two siblings from consecutive pregnancies *J Pediatr 77* 417 1970
8 Graham C B Thal A & Wassum C S Rubella like bone changes in congenital cytomegalic inclusion disease *Radiology 94* 39 1970
9 Hanshaw J B Congenital and aquired cytomegalo-virus infection *Pediatr Clin North Am 13* 279 1966
10 Krech U Complement fixing antibodies against cytomegalovirus in different parts of the world *Bull Wld Hlth Org 49* 103 1973
11 Krech U Konjajev Z & Jung M Congenital cytomegalovirus infection in siblings from consecu tive prgnancies *Hel Paediatr Acta* ?6 355 1971
1? Kumar M L Nankervis G A & Gold E Inap parent congenital cytomegalovirus infection *N Engl J Med* ?88 1370 1973

cifications in any of the infants and chest X rays give no evidence of cardial or pulmonary disease In two children (cases 4 and 6) X ray of the lower extremities showed pronounced osseous changes consisting of translucent spots interspersed between bands of sclerosis in and near the metaphyses In another (case 3) periostal coating of the long bone diaphyses was seen The lesions had healed at 8 months but with marked periostal reactions left in all three infants

DISCUSSION

With the diagnosis of congenital CMV infection documented by the presence of CMV in the urine before the age of 5 days we found an incidence of 0 4% in our population This incidence is lower than in any other studies based on the same diagnostic procedure (12 19 21) Reported incidences of 1–3 5% are from previous studies predominantly composed of young mothers and with the highest rate in neonates whose mothers are both young and recruited from low socio-economic populations The mean age of the parturient women in our study population was higher than in these studies and our affluent society will further reduce the low risk of congenital CMV infection in our child bearing women The general prevalence of CMV infection in fertile women was estimated on the basis of the presence of CF antibodies in cord sera as blood specimens from mothers were not available Our figures for seropositivity correspond well with those of other studies in populations comparable with ours (10) showing a high but decreasing percentage of susceptible women in the child bearing age Parallel to this a decrease in the number of congenitally infected infants was seen

The virus isolation procedure in which urines are pooled and cultured individually if necessary has been used with success in previous studies (20) In our hands this resource saving procedure also functioned satisfactorily To increase the sensitivity of the isolation procedure we used monolayers in bottles of 50 cm and it is our impression that the isolates were given the necessary growth conditions Thus our low incidence seems to be real and not caused by a deficient isolation procedure

The clinical findings in the infected infants in the present study agree with previous reports (4 5 13 15 20) and confirm the inapparent or mild manifestations of most congenital CMV infections found by screening Common to these investigations are that few or none of the infants had clinically recognizable illness in the immediate postnatal period specifically no symptoms from the brain or eyes which carry a poor prognosis for future psychomotor development Hanshaw has estimated the frequency of this discouraging form to be about 10% of infected neonates (13)

Follow up of the present infected children showed that the child with slight cerebral symptoms perinatally was doing well later on and the complicated delivery rather than CMV could be responsible for the symptoms

Series in which a high rate of unfavourable clinical outcome have been reported although the infants were asymptomatic at birth consisted of selected populations of young mothers from low socio-economic classes giving birth to a high percentage of premature infants (13 21) In many of these infants it is not possible to make any pathogenetic conclusions It can well be that the infection causes the prematurity or that the infection alone can be responsible for the sequelae observed In our infected premature twins without any objective signs of systemic CMV infection we are inclined to ascribe their retarded physical and psychomotor development to prematurity slight neonatal asphyxia and bad rearing

The diagnostic value of X ray examination of the long metaphyseal zones of the long bones is noteworthy The osseous changes first described by Graham et al (8) are not specific for CMV but are seen in other intra uterine infection

These latter diseases were not probable in our cases as the infants were seronegative for IgM rubella antibodies and the mothers were WR negative The prognostic value of deter mining virus excretion titres is uncertain There are differences between the infants both in the initial titres and the titres at follow up However later examination of the chil dren is needed to draw any conclusions as to the significance of this parameter Melish & Hanshaw (13) found a correlation between the presence of specific CMV IgM antibodies and severe systemic damage Four of our in fants had elevated levels of IgM antibodies but none had specific CMV IgM antibodies at birth

Foetal infection with CMV may occur both during a primary infection of the mother and in mothers who are immune during the whole pregnancy (19) The reason why some infants sustain severe damage while the majority of the infants are asymptomatic is unknown but it is most likely as in congenital rubella that a primary infection early in preg nancy may be most dangerous to the foetus (1 14) On the other hand examples of asymptomatic congenital infections in infants whose mothers had a serologically proven primary infection during pregnancy are known (19) On the basis of studies in siblings consecu tively infected in utero (7 11 19 23) and studies in mothers who were immune precon ceptionally (19) it seems justified to conclude that congenital infection in infants born of immune mothers are asymptomatic It is un known whether the transmission in both primary and reactivated infections occurs transplacentally during a viraemia or the foetus may be infected via an ascending infec tion from the cervix which is known to be a site of predilection for CMV during preg nancy (17)

A continued follow up of the present series will be performed but may not provide further information of the significance of congenital CMV infection with the strong impact environ ment has with increasing age on infant intel ligence and performance Starr et al reported that infants who were asymptomatic at birth and at one year of age did not either show any sensoneural impairment at four years (21) Our preliminary conclusion therefore is that the foetal CMV infection observed in our limited series is not of sufficient signifi cance to deserve routine screening for CMV infection or a vaccination programme in our population

ACKNOWLEDGEMENTS

The authors wish to thank Johannes Ipsen D M Sc M P H Professor of Epidemiology and Medical Statis tics University of Aarhus for valuable assistance in the planing of this study This work was supported by Statens Lægevidenskabelige Forskningsråd Grant No 51? 5704

REFERENCES

1 Altschuler G & McAdams A J Cytomegalic inclusion disease of a nineteen week fetus *Am J Obstet Gynecol 111* ?95 1971
2 Andersen H K & Spencer E S Cytomegalovirus infection among renal allograft recipients *Acta Med Scand 186* 7 1969
3 Andersen P & Andersen H K Immunoglobulin levels and specific viral antibodies in relation to smooth muscle antibodies in cytomegalovirus in fection *J Clin Lab Immunol* 1979 in press
4 Berenberg W & Nankervis G Long term follow up of cytomegalic inclusion disease of infancy *Pediat ics 46* 403 1970
5 Collaborative study Cytomegalovirus infection in the North West of England *Arch Dis Child 45* 513 1970
6 Elek S D & Stern H Development of a vaccine against mental retardation caused by cytomegalovirus infection in utero *Lancet I* 1 1974
7 Embil J A Ozere R L & Haldane E V Congenital cytomegalovirus infection in two siblings from consecutive pregnancies *J Pediatr 77* 417 1970
8 Graham C B Thal A & Wassum C S Rubella like bone changes in congenital cytomegalic inclusion disease *Radiology 94* 39 1970
9 Hanshaw J B Congenital and aquired cytomegalo virus infection *Ped atr Clin North Am 13* ?79 1966
10 Krech U Complement fixing antibodies against cytomegalovirus in different parts of the world *Bull Wld Hlth O g* 49 103 1973
11 Krech U Konjajev Z & Jung M Congenital cytomegalovirus infection in siblings from consecu tive prgnancies *Helv Paed atr Acta 26* 355 1971
1 Kumar M L Nankervis G A & Gold E Inap parent congenital cytomegalovirus infection *N Engl J Med 288* 1370 1973

cificitions in iny of the infints ind chest X rays gave no evidence of cardial or pulmonary disease In two children (cases 4 and 6) X ray of the lower extremities showed pronounced osseous changes consisting of translucent spots interspersed between bands of sclerosis in and near the metaphyses In another (case 3) periosteal coating of the long bone diaphyses was seen The lesions had healed at 8 months but with marked periosteal reactions left in all three infants

DISCUSSION

With the diagnosis of congenital CMV infection documented by the presence of CMV in the urine before the age of 5 days we found an incidence of 0 4% in our population This incidence is lower than in any other studies based on the same diagnostic procedure (12 19 21) Reported incidences of 1–3 5% are from previous studies predominantly composed of young mothers and with the highest rate in neonates whose mothers are both young and recruited from low socio-economic populations The mean age of the parturient women in our study population was higher than in these studies and our affluent society will further reduce the low risk of congenital CMV infection in our child bearing women The general prevalence of CMV infection in fertile women was estimated on the basis of the presence of CF antibodies in cord sera as blood specimens from mothers were not available Our figures for seropositivity correspond well with those of other studies in populations comparable with ours (10) showing a high but decreasing percentage of susceptible women in the child bearing age Parallel to this a decrease in the number of congenitally infected infants was seen

The virus isolation procedure in which urines are pooled and cultured individually if necessary has been used with success in previous studies (20) In our hands this resource saving procedure also functioned satisfactorily To increase the sensitivity of the isolation procedure we used monolayers in bottles of 50 cm² and it is our impression that the isolates were given the necessary growth conditions Thus our low incidence seems to be real and not caused by a deficient isolation procedure

The clinical findings in the infected infants in the present study agree with previous reports (4 5 13 15 20) and confirm the inapparent or mild manifestations of most congenital CMV infections found by screening Common to these investigations are that few or none of the infants had clinically recognizable illness in the immediate postnatal period specifically no symptoms from the brain or eyes which carry a poor prognosis for future psychomotor development Hanshaw has estimated the frequency of this discouraging form to be about 10% of infected neonates (13)

Follow up of the present infected children showed that the child with slight cerebral symptoms perinatally was doing well later on and the complicated delivery rather than CMV could be responsible for the symptoms

Series in which a high rate of unfavourable clinical outcome have been reported although the infants were asymptomatic at birth consisted of selected populations of young mothers from low socio economic classes giving birth to a high percentage of premature infants (13 21) In many of these infants it is not possible to make any pathogenetic conclusions It can well be that the infection causes the prematurity or that the infection alone can be responsible for the sequelae observed In our infected premature twins without any objective signs of systemic CMV infection we are inclined to ascribe their retarded physical and psychomotor development to prematurity slight neonatal asphyxia and bad rearing

The diagnostic value of X ray examination of the long metaphyseal zones of the long bones is noteworthy The osseous changes first described by Graham et al (8) are not specific for CMV but are seen in other intrauterine infections such as rubella and syphilis

These latter diseases were not probable in our cases as the infants were seronegative for IgM rubella antibodies and the mothers were WR negative The prognostic value of determining virus excretion titres is uncertain There are differences between the infants both in the initial titres and the titres at follow up However later examination of the children is needed to draw any conclusions as to the significance of this parameter Melish & Hanshaw (13) found a correlation between the presence of specific CMV IgM antibodies and severe systemic damage Four of our infants had elevated levels of IgM antibodies but none had specific CMV IgM antibodies at birth

Foetal infection with CMV may occur both during a primary infection of the mother and in mothers who are immune during the whole pregnancy (19) The reason why some infants sustain severe damage while the majority of the infants are asymptomatic is unknown but it is most likely as in congenital rubella that a primary infection early in pregnancy may be most dangerous to the foetus (1 14) On the other hand examples of asymptomatic congenital infections in infants whose mothers had a serologically proven primary infection during pregnancy are known (19) On the basis of studies in siblings consecutively infected in utero (7 11 19 23) and studies in mothers who were immune preconceptionally (19) it seems justified to conclude that congenital infection in infants born of immune mothers are asymptomatic It is unknown whether the transmission in both primary and reactivated infections occurs transplacentally during a viraemia or the foetus may be infected via an ascending infection from the cervix which is known to be a site of predilection for CMV during pregnancy (17)

A continued follow up of the present series will be performed but may not provide further information of the significance of congenital CMV infection with the strong impact environment has with increasing age on infant intelligence and performance Starr et al reported that infants who were asymptomatic at birth and at one year of age did not either show any sensoneural impairment at four years (21) Our preliminary conclusion therefore is that the foetal CMV infection observed in our limited series is not of sufficient significance to deserve routine screening for CMV infection or a vaccination programme in our population

ACKNOWLEDGEMENTS

The authors wish to thank Johannes Ipsen D M Sc M P H Professor of Epidemiology and Medical Statistics University of Aarhus for valuable assistance in the planing of this study This work was supported by Statens Lægevidenskabelige Forskningsråd Grant No 512-5704

REFERENCES

1 Altschuler G & McAdams A J Cytomegalic inclusion disease of a nineteen week fetus *Am J Obstet Gynecol 111* 295 1971
2 Andersen H K & Spencer E S Cytomegalovirus infection among renal allograft recipients *Acta Med Scand 186* 7 1969
3 Andersen P & Andersen H K Immunoglobulin levels and specific viral antibodies in relation to smooth muscle antibodies in cytomegalovirus infection *J Clin Lab Immunol* 1979 in press
4 Berenberg W & Nankervis G Long term follow up of cytomegalic inclusion disease of infancy *Ped atrics 46* 403 1970
5 Collaborative study Cytomegalovirus infection in the North West of England *Arch Dis Child 45* 513 1970
6 Elek S D & Stern H Development of a vaccine against mental retardation caused by cytomegalovirus infection in utero *Lancet I* 1 1974
7 Embil J A Ozere R L & Haldane E V Congenital cytomegalovirus infection in two siblings from consecutive pregnancies *J Pediatr 77* 417 1970
8 Graham C B Thal A & Wassum C S Rubella like bone changes in congenital cytomegalic inclusion disease *Radiology 94* 39 1970
9 Hanshaw J B Congenital and aquired cytomegalovirus infection *Pediat Clin North Am 13* 279 1966
10 Krech U Complement fixing antibodies against cytomegalovirus in different parts of the world *Bull Wld Hlth O g 49* 103 1973
11 Krech U Konjajev Z & Jung M Congenital cytomegalovirus infection in siblings from consecutive prgnancies *Helv Paed atr Acta 26* 355 1971
1 Kumar M L Nankervis G A & Gold E Inapparent congenital cytomegalovirus infection *N Engl J Med 288* 1370 1973

13 Melish F M & Hanshaw J B Congenital cytomegalovirus infection Developmental progress of infants detected by routine screening *Am J Dis Child 126* 190 1973
14 Monif G R G Egan E A Held B & Fitzman D V The correlation of maternal cytomegalovirus infection during varying stages in gestation with neonatal involvement *J Pediatr 80* 17 1972
15 Nakao T Chiba S Ohsaki M & Ikeda S Studies of cytomegalovirus infection in newborn infants *Tohoku J Exp Med 105* 191 1971
16 Plotkin S A Just M & Farquhar J Clinical trials of immunization with live human cytomegalovirus (Towne strain) *Pediatr Res 10* 392 1976
17 Reynolds D W Stagno S Stubbs K G Dahle A J Livingston M M Saxon S S & Alford C A Inapparent congenital cytomegalovirus infection with elevated cord IgM levels *N Engl J Med 290* 291 1974
18 Silva I M de Kampfner G L Lister C M & Tobin J O H Identification of pregnancies at risk from cytomegalovirus infection *J Hyg Camb 79* 347 1977
19 Stagno S Reynolds D W Huang E S Thames S D Smith R J & Alford C A Congenital cytomegalovirus infection Occurrence in an immune population *N Engl J Med 296* 1254 1977
20 Starr J G & Gold E Screening of newborn infants for cytomegalovirus infection *J Pediatr 73* 820 1968
21 Starr J G Bart R D & Gold E Inapparent congenital cytomegalovirus infection Clinical and epidemiological characteristics in early infancy *N Engl J Med 282* 1075 1970
22 Stern H & Tucker S M Prospective study of cytomegalovirus infection in pregnancy *Brit Med J 2* 268 1973
23 Yeager A S Martin H P & Stewart J A Congenital cytomegalovirus infection outcome for the subsequent sibling *Clin Pediatr 16* 45 1977

Submitted July 27 1978
Accepted Oct 28 1978

(H K A) Institute of Medical Microbiology
University of Aarhus
8000 Aarhus C
Denmark

Acta Pædiatr Scand 68 337–339 1979

NEONATAL SEPTICEMIA AND PERINATAL RISK FACTORS

G BERGQVIST M ERIKSSON and R ZETTERSTRÖM

From the Department of Paediatrics Karolinska Institutet
St Goran s Children s Hospital Stockholm Sweden

ABSTRACT Bergq ist G Eriksson M and Zetterstrom R (Department of Paediatrics Karolinska Institutet St Goran s Children s Hospital Stockholm Sweden) Neonatal septi cemia and perinatal risk factors Acta Paediatr Scand 68 337 1979 —Many methods for screening and prediction of neonatal septicemia have been tried In this study a score re lated to both perinatal risk factors and neonatal diseases was tested upon healthy newborn infants infants with septicemia and infants with other diseases Statistical differences were found between infants with neonatal septicemia and infants with other neonatal diseases as well as normal newborns It was also possible to find a relationship between certain pre disposing factors and predominance of certain pathogens Complications during pregnancy and delivery were most often found in the group B streptococcal combinations of invasive procedures and neonatal diseases in the staphylococcal group and surgical procedures in the gram negative group

KEY WORDS Newborn septicemia group B streptococcus gram negative bacteria staphylococcus

Neonatal septicemia is still a serious condition with attendant high mortality (1 4 5) In nu merous retrospective studies certain predis posing factors related to pregnancy delivery as well as neonatal diseases have been identi fied (4 14) In recent years attention has also been drawn to the risk of cross infections in neonatal intensive care units (6 7 11)

In order to make the earliest possible diag nosis in neonatal septicemia several screen ing procedures have been tried often in com bination with cultures from various sites (8 9 13) In one study on infants born following premature rupture of the membranes it was shown that babies with high risk scores related to pregnancy and delivery ran a significantly greater risk of developing septicemia (13)

However no studies have attempted to combine the scores of risk factors related to pregnancy delivery and procedures used in the care of the infant and relate them to the different causative organisms

This study was undertaken to evaluate a scoring method in different groups of newborn infants Moreover the scores were related to the causative organism found in those infants having neonatal septicemia

MATERIALS AND METHODS

One hundred and five consecutive newborn infants exam ined in the maternity ward and 49 consecutive babies admitted to a neonatal unit under different diagnoses were scored The scores were compared with those of 61 con secutive infants less than ? weeks of age cared for at St Goran s Children s Hospital who had neonatal septi cemia as evidenced by clinical symptoms and at least one positive blood culture obtained from a peripheral vein Of these 61 infants 18 were surgical patients The infants with neonatal septi emia were born during the years 1970–73 and the other babies in 1973 The incidence of neona tal septicemia has been calculated as 1 4 per 1000 new borns The infants were given a score of 1 for each of the following 10 items maternal disease e g diabetes severe toxicemia infection rupture of the membranes more than 24 hours before the baby was born foul smelling amniotic fluid complicated delivery e g emergency forceps va cuum extraction and section preterm birth (gest age <37 weeks) small for gestational age Apgar score <7 at 1 minute umbilical catheterization respiratory distress and other neonatal diagnoses e g congenital malformation leading to operative procedures and later long periods of i v fluid nutrition

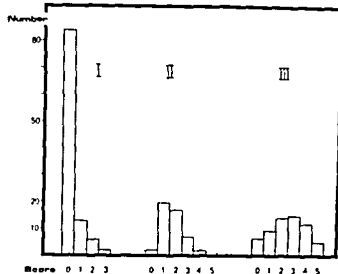


Fig 1 Neonatal score rates in the three groups of infants which have been studied I 105 consecutive newborn infants II 49 newborn infants consecutively admitted to a pediatric department III 61 cases with neonatal septicemia

For these infants the mother s breast milk or pasteurized milk from other mothers has been the rule except for very sick infants and infants with intestinal malformations where i v fluid and parenteral nutrition were given

For statistical analysis the Kruskal & Wallis multi sample test was used (3)

In 133 consecutive infants with neonatal septicemia (including the 61 infants that were scored) the total number of perinatal risk factors were grouped according to the four causative organisms *Staphylococcus aureus* group B streptococcus *E coli* and gram negative rods other than *E coli*

RESULTS

The scoring profiles of the different groups are shown in Fig 1 There is a significant difference between infants with septicemia and other infants admitted to the neonatal unit The relation between the number of perinatal risk factors and the causative organism is shown in Table 1 As can be seen a combination of several risk factors related to pregnancy delivery and procedures performed before the diagnosis is commonly associated with three of the causative organisms while complications during pregnancy and delivery are more often associated with infections caused by group B streptococci In cases in which there is only one factor related to a procedure before the diagnosis exchange transfusion dominates in the staphylococcal group and surgical procedures in the gram negative group Umbilical vessel catheters are common in the staphylococcal and gram negative groups

DISCUSSION

This study shows that infants who develop septicemia usually have a combination of several predisposing factors They have a significantly higher score than normal newborns and sick newborns who do not develop septicemia A score of 3 or more was found in 52% of the total septicemia group but in only 20% of the newborns admitted to a neonatal ward and in 2% of consecutive healthy newborns With a slightly different scoring system putting more emphasis on pregnancy and delivery Takkar et al (13) found that the risk of developing neonatal septicemia rose from 20% with a score of 4 up to 60% with a score of 6 As in our study they too found a few infants with low scores who later developed septicemia

Some differences were noted on analysis of the relation between the predisposing factors and the causative organisms Risk factors related to pregnancy and delivery such as maternal infection and premature rupture of the membrane were common in group B streptococcal infections while additional risk factors were common in gram negative infections Several other investigators have also shown that especially the early onset type of group B streptococcal disease is combined with complications during pregnancy and delivery such as early rupture of the membranes even though nosocomial spread also seems to exist (2) Differences between streptococcal and other neonatal infections have also recently been reported as regards age at onset and mortality (10)

A combination of several risk factors was most often found in staphylococcal and gram negative infections The increased incidence of so called nosocomial infections in neonatal intensive care units has attracted increasing at

Table 1 *Relation between the number of perinatal risk factors and the causative organism in 133 infants with neonatal septicemia*

Etiology	Total (*n*)	Only one factor related to: A) Pregnancy and Delivery (*n*)	Only one factor related to: B) Neonatal procedures before Diagnosis (*n*)	More than one factor related to: A) Complications during Pregnancy and Delivery (*n*)	More than one factor related to: B) Neonatal procedures before Diagnosis (*n*)	Combinations of A and B (*n*)	No factor (*n*)
Staphylococcus aureus	39	3	5	1	1	23	6
Group B streptococcus	25	13	0	5	0	[illegible]	5
E coli	38	3	6	7	2	14	6
Gram negative organisms other than *E coli*	30	0	[illegible]	0	4	23	1

tention in recent years and has been shown to be present in 25% of the patients (6 7 11) This could be related both to host factors such as low birth weight and environmental factors It has also been demonstrated that various changes in the ward routine will affect the predominance of bacteria isolated discontinuation of bathing in hexachlorophene increased the rate of colonization with staphylococci and procedures to reduce this rate instead increased the incidence of gram negative organisms (7 12)

Beside analysing the host factor of birth weight we have also studied other factors such as invasive procedures and found a relationship to the causative organism i e permanent catheters were more commonly associated with staphylococcal infections and operative procedures and parenteral nutrition with gram negative infections

Prospective studies will be needed not only on infected infants but also on colonization to confirm the relationship between certain predisposing factors and predominance of certain pathogens that we have found

REFERENCES

1 Albppan L C & Andrews B F Neonatal sepsis *Clin Pediat* 14 381 1975
2 Baker C J Summary of the workshop on perinatal infections due to group B streptococcus *J Infect Dis* 136 137 1977
3 Breadley J V *Dist ub-free Statistical Tests* Prentice H II New Jersey 1968 p 1 9
4 Davies P A Bacterial infection in the foetus and newborn *Arch Dis Child* 46 1 1971
5 Gotoff S P & Behrman R E Neonatal septicemia *J Pediatr* 76 142 1970
6 Hable K A Matsen J M Wheeler D J Hunt C E & Quie P G Klebsiella type 33 septicemia in an infant intensive care unit *J Pediatr* 80 970 1972
7 Hemming V G Overall J C & Britt M R Nosocomial infections in a newborn intensive care unit *N Engl J Med* 294 1310 1976
8 Higgs S C Malan A F & De V Heese H The perinatal infective environment and infants of very low birth weight *S Afr Med J* 51 621 1977
9 Hosmer M E & Sprunt K Screening method for identification of infected infant following premature rupture of maternal membranes *Pediatrics* 49 283 1972
10 Jeffery H Mitchison R Wigglesworth J S & Davies P A Early neonatal bacteraemia A comparison of group B streptococcal other gram positive and gram negative infections *Arch Dis Child* 52 683 1977
11 Morehead C D & Houck P W Epidemiology of pseudomonas infections in a pediatric intensive care unit *Am J Dis Child* 124 564 1972
12 Light I J Sutherland J M Cochran L & Sutorius J Ecologic relation between staphylococcus aureus and pseudomonas in a nursery population *N Engl J Med* 278 1 43 1968
13 Takkar P Bhakoo O N & Narang A Scoring system for the prediction of early neonatal infections *Indian Pediatr* 11 597 1975
14 Tyler C W & Albers W H Obstetric factors related to bacteremia in the newborn infant *Am J Obstet Gynecol* 94 970 1966

Submitted July 14 1978
Accepted Sept 14 1978

(M E) Department of Paediatrics
St Görans Sjukhus
S 11 81 Stockholm
Sweden

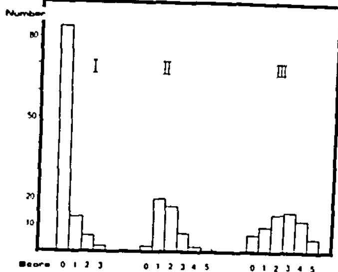


Fig 1 Neonatal score rates in the three groups of infants which have been studied I 105 consecutive newborn infants II 49 newborn infants consecutively admitted to a pediatric department III 61 cases with neonatal septicemia

For these infants the mother s breast milk or pasteurized milk from other mothers has been the rule except for very sick infants and infants with intestinal malformations where i v fluid and parenteral nutrition were given

For statistical analysis the Kruskal & Wallis multi sample test was used (3)

In 133 consecutive infants with neonatal septicemia (including the 61 infants that were scored) the total number of perinatal risk factors were grouped according to the four causative organisms *Staphylococcus aureus* group B streptococcus *E coli* and gram negative rods other than *E coli*

RESULTS

The scoring profiles of the different groups are shown in Fig 1 There is a significant difference between infants with septicemia and other infants admitted to the neonatal unit The relation between the number of perinatal risk factors and the causative organism is shown in Table 1 As can be seen a combination of several risk factors related to pregnancy delivery and procedures performed before the diagnosis is commonly associated with three of the causative organisms while complications during pregnancy and delivery are more often associated with infections caused by group B streptococci In cases in which there is only one factor related to a procedure before the diagnosis exchange transfusion dominates in the staphylococcal group and surgical procedures in the gram negative group Umbilical vessel catheters are common in the staphylococcal and gram negative groups

DISCUSSION

This study shows that infants who develop septicemia usually have a combination of several predisposing factors They have a significantly higher score than normal newborns and sick newborns who do not develop septicemia A score of 3 or more was found in 52% of the total septicemia group but in only 20% of the newborns admitted to a neonatal ward and in 2% of consecutive healthy newborns With a slightly different scoring system putting more emphasis on pregnancy and delivery Takkar et al (13) found that the risk of developing neonatal septicemia rose from 20% with a score of 4 up to 60% with a score of 6 As in our study they too found a few infants with low scores who later developed septicemia

Some differences were noted on analysis of the relation between the predisposing factors and the causative organisms Risk factors related to pregnancy and delivery such as maternal infection and premature rupture of the membrane were common in group B streptococcal infections while additional risk factors were common in gram negative infections Several other investigators have also shown that especially the early onset type of group B streptococcal disease is combined with complications during pregnancy and delivery such as early rupture of the membranes even though nosocomial spread also seems to exist (2) Differences between streptococcal and other neonatal infections have also recently been reported as regards age at onset and mortality (10)

A combination of several risk factors was most often found in staphylococcal and gram negative infections The increased incidence of so called nosocomial infections in neonatal intensive care units has attracted increasing at

Table 1 *Relation between the number of perinatal risk factors and the causative organism in 133 infants with neonatal septicemia*

		Only one factor related to		More than one factor related to			
Etiology	Total (*n*)	A) Pregnancy and Delivery (*n*)	B) Neonatal procedures before Diagnosis (*n*)	A) Complications during Pregnancy and Delivery (*n*)	B) Neonatal procedures before Diagnosis (*n*)	Combinations of A and B (*n*)	No factor (*n*)
Staphylococcus aureus	39	3	5	1	1	23	6
Group B streptococcus	25	13	0	5	0	2	5
E coli	38	3	6	7	2	14	6
Gram negative organisms other than *E coli*	30	0	2	0	4	23	1

tention in recent years and has been shown to be present in 25% of the patients (6 7 11) This could be related both to host factors such as low birth weight and environmental factors It has also been demonstrated that various changes in the ward routine will affect the predominance of bacteria isolated discontinuation of bathing in hexachlorophene increased the rate of colonization with staphylococci and procedures to reduce this rate instead increased the incidence of gram negative organisms (7 12)

Beside analysing the host factor of birth weight we have also studied other factors such as invasive procedures and found a relationship to the causative organism i e permanent catheters were more commonly associated with staphylococcal infections and operative procedures and parenteral nutrition with gram negative infections

Prospective studies will be needed not only on infected infants but also on colonization to confirm the relationship between certain predisposing factors and predominance of certain pathogens that we have found

REFERENCES

1 Akjpan L C & Andrews B F Neonatal sep is *Clin Pediatr 14* 181 1975

Baker C J Summary of the workshop on perinatal infections due to group B streptococcus *J Infect Dis 136* 137 1977

3 Breadley J V *D st ub-f ee Statistical Tests* Prentice Hall New Jersey 1968 p 1?9

4 Davies P A Bacterial infection in the foetus and newborn *Arch Dis Child 46* 1 1971

5 Gotoff S P & Behrman R E Neonatal septicemia *J Pediatr 76* 14? 1970

6 Hable K A Matsen J M Wheeler D J Hunt C E & Quie P G Klebsiella type 33 septicemia in an infant intensive care unit *J Pediatr 80* 9?0 197?

7 Hemming V G Overall J C & Britt M R Nosocomial infections in a newborn intensive care unit *N Engl J Med 294* 1310 1976

8 Higgs S C Malan A F & De V Heese H The perinatal infective environment and infants of very low birth weight *S Afr Med J 51* 6?1 1977

9 Hosmer M E & Sprunt K Screening method for identification of infected infant following premature rupture of maternal membranes *Pediatrics 49* ?83 197?

10 Jeffery H Mitchison R Wigglesworth J S & Davies P A Early neonatal bacteraemia A comparison of group B streptococcal other gram positive and gram negative infections *Arch Dis Child 52* 683 1977

11 Morehead C D & Houck P W Epidemiology of pseudomonas infections in a pediatric intensive care unit *Am J Dis Child 124* 564 197?

12 Light I J Sutherland J M Cochran L & Sutorius J Ecologic relation between staphylococcus aureus and pseudomonas in a nursery population *N Engl J Med 278* 1 43 1968

13 Takkar P Bhakoo O N & Narang A Scoring system for the prediction of early neonatal infections *Indian Pediatr 11* 597 1975

14 Tyler C W & Albers W H Obstetric factors related to bacteremia in the newborn infant *Am J Obstet Gynecol 94* 970 1966

Submitted July 14 1978
Accepted Sept 14 1978

(M E) Department of Paediatrics
S t Görans Sjukhus
S-112 81 Stockholm
Sweden

Acta Pædiatr Scand 68 341–344 1979

DIAGNOSTIC VALUE OF SYMPTOMS AND CLEAN VOIDED URINE SPECIMEN IN CHILDHOOD URINARY TRACT INFECTION

J PYLKKANEN J VILSKA and O KOSKIMIES

From the Children s Hospital University of Helsinki Finland

ABSTRACT Pylkkanen J Vilska J and Koskimies O (The Children s Hospital University of Helsinki Helsinki Finland) Diagnostic value of symptoms and clean voided urine specimen in childhood urinary tract infection Acta Paediatr Scand 68 341 1979 —In diagnosing urinary tract infection (UTI) the symptoms of 477 infants and children and the findings in their clean voided urine specimens were evaluated 322 patients were considered infected when a bacterial culture of suprapubic aspirate was used as a diagnostic reference No diagnosis was attempted on the basis of symptoms only Numerous bacteria or ≥200 leuc /mm^3 in an uncentrifuged clean voided urine specimen or ≥10^5 bact /ml in quantitative bacterial culture were found in 59 % 42 % and 81 % of the infected symptomatic patients The diagnostic accuracies of these indices were 88 % 94 % and 95 % respectively In asymptomatic patients the accuracies were considerably lower Among these infected patients normal or equivocal isolated findings in the clean voided urine specimens were frequently seen and could not markedly be reduced by the various related factors such as technique of urine collection urine specific gravity or pH of urine None of the above mentioned indices of the clean voided urine specimens seems to be alone accurate and sensitive enough for diagnosing UTI and therefore these should be used in combination The advantage of immediately obtaining results supports the use of urine microscopy as a primary diagnostic method in symptomatic UTI of childhood in particular

KEY WORDS Urinary tract infection suprapubic bladder aspiration children

Suprapubic aspiration of urine (SPA) is the most reliable way to diagnose or exclude child hood urinary tract infection (UTI) (1 4) However UTI is usually suspected on the basis of its symptoms and diagnosed by analysing clean voided urine specimen (CVUS) We evaluated the symptoms the counts of leucocytes and bacteria on microscopy and the quantitative bacterial cultures from the uncentrifuged CVUSs and used the bacterial culture from SPA as a diagnostic reference The effect of the various related factors on the findings in the CVUSs was also investigated

PATIENTS AND METHODS

477 patients (164 infants and 313 children) seen at the general outpatient clinic were included in the study On the basis of the findings in their SPA 322 patients (74 infants and 248 children) were shown to have UTI Of these 477 patients [illegible]7 had symptoms suggesting acute UTI SPA was also performed on [illegible]05 asymptomatic patients because of a high leucocyte count in or an abnormal quantitative bacterial culture from their CVUSs

Techniques of urine collection SPA was performed as described by Saccharov & Pryles (8) No complications other than occasional microscopic hematuria were seen Both CVUS and SPA were always obtained within the same 24 hour period Before collecting CVUS the external genitalia were gently cleansed with 0 05% Chlorhexidine solution then rinsed several times with water and dried Coloplast® urine collection bags (Espergærde Denmark) were used on the infants The bag was changed every hour until a sample was voided The collection of mid stream samples from the children was performed as described by Huttunen et al (6)

Analysis of the CVUSs Immediately after collection the well mixed CVUSs were examined under microscope for leucocyte and bacterial count using a Burker counting chamber A sample was also obtained to carry out quantitative bacterial culture (Uricult® Orion Pharmaceutical Co Helsinki) The amounts of leucocytes were grouped ≤10 11–199 or ≥200 leuc /mm The numbers of bacteria in the native preparation were expressed as nil scanty or numerous (≥30 bact /microscopic field) The bacterial cultures were classified ≤10^3 10^4 or ≥10^5 bact /ml

Table 1. *Principal complaints suggesting UTI*

200 children with suspect first UTI. Every patient may have one or several leading symptoms

Age (years) Bacterial culture from SPA	<1.0 +/−	1.0–2.9 +/−	≥3.0 +/−
Enuresis	0/0	6/2	4/0
Frequent micturition	3/3	10/7	14/6
Painful micturition	7/5	17/8	23/8
Bad smell of urine	5/0	3/0	0/0
Blood in urine	1/0	5/2	3/0
Vomiting	15/8	5/6	8/7
Abdominal pain	3/0	2/3	24/14
Diarrhoea	6/5	5/0	2/2
Obstipation	2/0	0/1	0/0
Back pain	0/0	1/0	2/1
Convulsions	6/1	1/2	0/2
Fever alone	11/8	2/0	0/1
Number of patients	49/26	30/24	48/23

Diagnosis of UTI. The SPAs were cultured on both Uricult® dipslides and blood agar plates. The patient was considered infected (SPA positive) when both cultures showed consistent bacterial growth. In 6 cases the plates or the slides had 1–2 occasional colonies considered to be due to aerial contamination.

Calculations. The chi square test was employed in comparing the proportions of the CVUS findings.

RESULTS

Value of symptoms in diagnosing UTI

200 patients were suspected to have their first symptomatic UTI and 127 of them were SPA positive. The principal complaints among the infected and non infected children are listed in Table 1. The same symptoms were found in both groups. The only slight difference was that the 8 patients whose mothers had observed bad smell of urine belonged to the infected group.

CVUS findings in diagnosing UTI

Table 2 shows the results from the CVUSs from the 322 infected and 155 non infected patients. All isolated leucocyte or bacterial findings on microscopy and in the CVUS bacterial cultures were detected in both groups.

Table 3 shows the diagnostic sensitivity and accuracy of the various indices of the CVUS. Of the symptomatic patients with UTI 59% had ≥200 leuc./mm³. On the other hand 88% of the children with this finding had UTI. The sensitivity and accuracy figures for numerous bacteria in the native preparations were 42% and 94% and for the bacterial culture of $\geq 10^5$ bact./ml in symptomatic patients 81% and 95% respectively. The corresponding diagnostic accuracies in asymptomatic patients were markedly lower. When the diagnostic limits of the indices were lowered to attain higher sensitivity the accuracies also became poorer.

The leucocyte counts showed no statistically significant relation to the other findings in the CVUSs. Instead 96% of the patients with numerous bacteria on microscopy had $\geq 10^5$ bact./ml in quantitative urine culture. Of the children with $\leq 10^3$ bac./ml 48% had numerous bacteria while 15% had no bacteria microscopically.

Effect of various related factors on CVUS findings

Since normal and equivocal findings in the CVUSs were frequently detected in the

Table 2. *Findings in uncentrifuged CVUSs*

322 infected (SPA positive) and 155 non infected patients

Bacterial culture from SPA	Symptomatic patients +/−	Asymptomatic patients +/−
Leucocyte count (cells/mm³)		
≥200	117/16	36/12
11–199	61/34	52/35
≤10	21/23	35/35
Number of bacteria		
Numerous	83/5	64/6
Scanty	89/20	42/24
Nil	27/48	17/52
Quantitative bacterial culture (bact./ml)		
$\geq 10^5$	161/9	109/41
10^4	18/9	6/7
$\leq 10^3$	20/55	8/34
Number of patients	199/73	123/82

Table 3 *Diagnostic sensitivity (S) and accuracy (A) of various isolated findings in uncentrifuged CVUSs*

3[illegible] infected (SPA positive) and 1[illegible]5 non infected patients

	Symptomatic patients (N=27[illegible])		Asymptomatic patients (N=[illegible]05)	
	S	A	S	A
Leucocyte count (cells/mm³)				
≥ 00	59%	88%	[illegible]9%	75%
≥11	89%	78%	7[illegible]%	65%
Number of bacteria				
Numerous	4[illegible]%	94%	5[illegible]%	91%
Scanty or numerous	86%	87%	86%	78%
Quantitative bacterial culture (bact /ml)				
≥10⁵	81%	95%	89%	73%
≥10	90%	89%	93%	70%

patients with UTI the effect of 5 anamnestic and 4 technical factors on leucocyte count on number of bacteria on microscopy and on quantitative bacterial culture were separately analysed in the infected patients No significant interaction between the related factors emerged Only the effects with a possible essential relevance are reported here

Anamnestic correlations

Sex and age of the patients had no significant effect on the CVUS findings *Presence or absence of symptoms* (Table 2) the leucocyte counts of the symptomatic patients were higher than those of the asymptomatic children ($p<0.005$) *Sequence number of UTIs* of the 48 patients with their first symptomatic UTI (age≥3 years) 71% had ≥200 leuc /mm³ and in 4% the leucocyte count was normal whereas for the 69 patients with recurrent symptomatic UTI (age ≥3 years) these frequencies were 41% and 17% respectively ($p<0.02$) *Concurrent antibiotic therapy* 19 patients with UTI received antibiotic therapy for upper respiratory infection 15 of them had ≥10⁵ bact /ml in the CVUS culture

Technical correlations

Technique of urine collection under microscope numerous bacteria were found in 29% and no bacteria in 27% of 76 bag specimens from the infected infants while in the mid stream samples this was the case in 51% and 10% of 246 patients ($p<0.005$) *Urine specific gravity* and *pH of urine* as well as *sequence number of* the CVUSs had no significant relation to the findings in the CVUSs Low urine specific gravity (≤1 004) and low pH of urine (pH≤5 0) were seen in 13% and 5% of the infected patients respectively

DISCUSSION

The diagnostic accuracy of childhood UTI is apparently far from satisfactory (1 2) The widely accepted use of urine sediment (3) is one of the causes of confusion pointed out by Houston (5) In order to clarify the diagnostic criteria of UTI we compared the symptoms and the findings of uncentrifuged CVUS with the bacterial culture from SPA

Certain symptoms are thought to suggest UTI in children In this study the infected and non infected patients could not be discriminated on the basis of their symptoms 73 of the 200 children with suspect symptomatic UTI had sterile SPASs Thus a reliable diagnosis of UTI cannot be made without urine analysis

In a typical case of UTI numerous bacteria and ≥200 leuc /mm³ are microscopically detected in uncentrifuged CVUSs and ≥10⁵ bact /ml are found in urine culture Nevertheless each one of these isolated findings was frequently seen in the urine of non infected patients Equivocal or even normal findings were not uncommon among the infected patients e g low leucocyte counts were especially found in recurrent and asymptomatic UTIs These findings did not show any distinct relation to the various technical factors such as technique of urine collection urine specific gravity and pH of urine

Table 1. *Principal complaints suggesting UTI*

200 children with suspect first UTI. Every patient may have one or several leading symptoms

Age (years) Bacterial culture from SPA	<1.0 +/−	1.0–2.9 +/−	>3.0 +/−
Enuresis	0/0	6/2	4/0
Frequent micturition	3/3	10/7	14/6
Painful micturition	7/5	17/8	23/8
Bad smell of urine	5/0	3/0	0/0
Blood in urine	1/0	5/2	3/0
Vomiting	15/8	5/6	8/7
Abdominal pain	3/0	2/3	24/14
Diarrhoea	6/5	5/0	2/2
Obstipation	2/0	0/1	0/0
Back pain	0/0	1/0	2/1
Convulsions	6/1	1/2	0/2
Fever alone	11/8	2/0	0/1
Number of patients	49/26	30/24	48/23

Diagnosis of UTI. The SPAs were cultured on both Uricult[a] dipslides and blood agar plates. The patient was considered infected (SPA positive) when both cultures showed consistent bacterial growth. In 6 cases the plates or the slides had 1–2 occasional colonies considered to be due to aerial contamination.

Calculations. The chi square test was employed in comparing the proportions of the CVUS findings.

RESULTS

Value of symptoms in diagnosing UTI

200 patients were suspected to have their first symptomatic UTI and 127 of them were SPA positive. The principal complaints among the infected and non infected children are listed in Table 1. The same symptoms were found in both groups. The only slight difference was that the 8 patients whose mothers had observed bad smell of urine belonged to the infected group.

CVUS findings in diagnosing UTI

Table 2 shows the results from the CVUSs from the 322 infected and 155 non infected patients. All isolated leucocyte or bacterial findings on microscopy and in the CVUS bacterial cultures were detected in both groups.

Table 3 shows the diagnostic sensitivity and accuracy of the various indices of the CVUS. Of the symptomatic patients with UTI 59% had ≥200 leuc./mm³. On the other hand 88% of the children with this finding had UTI. The sensitivity and accuracy figures for numerous bacteria in the native preparations were 42% and 94% and for the bacterial culture of ≥10⁵ bact./ml in symptomatic patients 81% and 95% respectively. The corresponding diagnostic accuracies in asymptomatic patients were markedly lower. When the diagnostic limits of the indices were lowered to attain higher sensitivity the accuracies also became poorer.

The leucocyte counts showed no statistically significant relation to the other findings in the CVUSs. Instead 96% of the patients with numerous bacteria on microscopy had ≥10⁵ bact./ml in quantitative urine culture. Of the children with ≤10⁵ bac./ml 48% had numerous bacteria while 15% had no bacteria microscopically.

Effect of various related factors on CVUS findings

Since normal and equivocal findings in the CVUSs were frequently detected in the

Table 2. *Findings in uncentrifuged CVUSs*

322 infected (SPA positive) and 155 non infected patients

Bacterial culture from SPA	Symptomatic patients +/−	Asymptomatic patients +/−
Leucocyte count (cells/mm³)		
≥200	117/16	36/12
11–199	61/34	52/35
≤10	21/23	35/35
Number of bacteria		
Numerous	83/5	64/6
Scanty	89/20	42/24
Nil	27/48	17/52
Quantitative bacterial culture (bact./ml)		
≥10⁵	161/9	109/41
10⁴	18/9	6/7
≤10³	20/55	8/34
Number of patients	199/73	123/82

Acta Pædiatr Scand 68 345–350 1979

MITRAL AND TRICUSPID VALVE VEGETATIONS IN INFANCY DIAGNOSED BY ECHOCARDIOGRAPHY

NILS-RUNE LUNDSTRÖM and GUDRUN BJÖRKHEM

From the Department of Paediatrics University Hospital Lund Sweden

ABSTRACT Lundstrom N R and Bjorkhem G (Department of Paediatrics University Hospital Lund Sweden) Mitral and tricuspid valve vegetations in infancy diagnosed by echocardiography Acta Paediatr Scand 68 345 1979 —The use of echocardiography in diagnosing intra-cardiac vegetations is described in two infants The condition is unusual in infancy but can be suspected from the clinical picture and positive blood cultures Using echocardiography it is possible to localize the vegetations exactly and estimate the extent of valvular involvement In one of the cases presented a vegetation on the anterior mitral leaflet was diagnosed and a gradual increase in size could be demonstrated In the other case a large echo-producing mass was found attached to the tricuspid valve protruding into the right ventricular outflow tract This mass represented a fungal vegetation In both cases autopsy later verified the echocardiographic findings

KEY WORDS Two-dimensional echocardiography cross-sectional echocardiography bacterial endocarditis fungal endocarditis

The diagnosis of bacterial or fungal endocarditis is usually based on clinical findings combined with positive blood cultures At autopsy one main finding is bacterial or fungal valvular vegetations Echocardiography has already been shown to be useful in demonstrating the valvular vegetations in infective endocarditis (4 6 8 11 12 14 17) The condition is unusual in infancy (10) and we report here 2 infants in whom echocardiography demonstrated vegetations on the mitral and tricuspid valves respectively

CASE REPORTS

Case 1

A newborn boy was admitted to the department of pediatric surgery at the age of one day because of vomiting No signs of intestinal obstruction were found At the age of one week the patient developed a low grade fever and muscular hypotonia Two days later he was transferred to the department of pediatrics On admission a septicaemia was suspected clinically and there were no signs of heart disease Blood and urine cultures revealed growth of plasma-coagulating staphylococci which was treated with appropriate antibiotics Within a few days the fever disappeared and the infant was well and blood cultures were negative Two weeks later the infant suddenly developed signs of severe congestive heart failure Auscultation revealed a high frequency pansystolic murmur at the apical region A chest X ray revealed left atrial and right ventricular enlargement Repeat echocardiographic examinations were performed and the results will be reported below Despite vigorous treatment (antibiotics digitalis diuretics) the condition of the patient deteriorated and he died at the age of 6 weeks 11 days after the onset of congestive heart failure

Autopsy The heart was grossly dilated but there was no evidence of congenital heart disease The anterior mitral leaflet was to a large extent destroyed and replaced by a large vegetation 8 mm in diameter The posterior mitral leaflet was normal

Echocardiography The examinations were performed with a standard ultrasonoscope (Ekoline 20 Smith Kline) and a 2.25 MHz unfocused transducer using standard techniques (13) Great care was taken to use the same gain settings at the repeat examinations

The first echocardiographic examination was performed on the day of the onset of congestive heart failure (Day 1) The echo from the anterior mitral leaflet showed a normal amplitude and pattern of movement but was unusually thick and partly with a fuzzy indistinct appearance (Fig 1 A) The echo from the posterior mitral leaflet was normal The left atrium and the right ventricle appeared enlarged (Table I)

A repeat echocardiographic examination three days

This work was supported by grants from the Swedish National Association against Heart and Chest disease and from "Förenade Liv" Mutual Group Life Insurance Company Stockholm Sweden

We think that none of the indices applied in this study gives alone sufficient sensitivity and accuracy in diagnosing UTI In particular we observed significant bacteriuria (7) in the CVUS of only 81% of the symptomatic and 89% *of the asymptomatic infected patients* In our opinion the use of Chlorhexidine solution did not influence these figures since the external genitalia were also rinsed with water several times and dried On the other hand false positive cultures were seen 10% of the symptomatic and even in 50% of the asymptomatic non infected children

Thus the separate findings of one or even several CVUSs should be combined for the diagnosis and SPA is needed in cases with diagnostic difficulties On microscopy the presence of numerous bacteria in an uncentrifuged CVUS predicted with great certainty (96%) $\geq 10^5$ bact /ml in the urine culture This great advantage of immediate results *supports the use of urine microscopy* as a primary diagnostic method for UTI in severely ill patients in particular Of course quantitative urine culture is necessary for confirming the diagnosis and for assessing antibiotic sensitivity

ACKNOWLEDGEMENT

This study was supported by the Foundation of Pediatric Research Sigrid Juselius Foundation and Orion Pharmaceutical Co

REFERENCES

1 Aronson A S Gustafson B & Svenningsen N W Combined suprapubic aspiration and clean voided urine examination in infants and children *Acta Paediatr Scand 63* 396 1973

2 *Bergvall B & Ljungblom B Å* Suprapubic aspiration of the urinary bladder for accurate diagnosis of urinary tract infections in children *Acta P e[illegible] Scand 61* 390 1972

3 Dolan T F Jr & Meyers A *A survey of office* management of urinary tract infections in childhood *Pediatrics 52* 21 1973

4 Edelmann C M Jr Ogwo J E Fine B P Martinez A B The prevalence of bacteriuria in full term and premature newborn infants *J Paed 82* 125 1973

5 *Houston I B Measurement of pyuria in urinary* tract infections *Arch Dis Child 44* 480 1969

6 Huttunen N P Makela P & Mella E Simple method for increasing reliability in diagnosis of urinary infection *Lancet I* 22 1970

7 Kass E H Asymptomatic infections of the urinary tract *Trans Ass Am Phys 69* 56 1956

8 *Saccharow L & Pryles C V* Further experience with the use of percutaneous suprapubic aspiration of the urinary bladder Bacteriological studies in 6[illegible] infants and children *Pediatrics 43* 1018 1969

Submitted June 29 1978
Accepted Oct 30 1978

(J P) The Children s Hospital
University of Helsinki
Stenbackinkatu 11
SF 00290 Helsinki 29
Finland

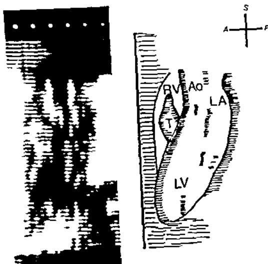


Fig 2 Two-dimensional echocardiogram (still frame from cine film) obtained with the transducer along the long axis of the heart together with schematic drawing showing the tumor mass (*T*) in the right ventricle (*RV*) *Ao*= aortic root *LA*=left atrium *LV*=left ventricle *A*= anterior *P*=posterior *S*=superior *I*=inferior

During three weeks of treatment the boy first improved a little but then slowly deteriorated Cultures from blood and urine were negative during treatment but since the child did not improve and the echocardiographic examinations still showed the same large mass in the right ventricle surgery was considered necessary At operation using cardio-pulmonary bypass the right atrium was first opened and a large vegetation was found extending from the base of the septal tricuspid leaflet which was partially destroyed As much as possible of the mass was removed through the right atrium and the right ventricle was then opened so that the main part of the mass could be reached and extirpated (Fig 3)

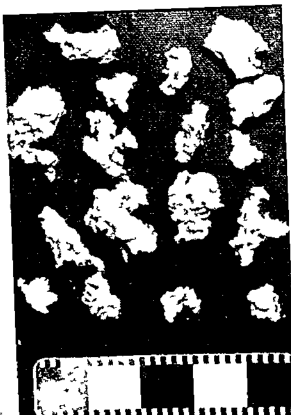


Fig 3 Photograph of the candida vegetations removed from the right ventricle during operation on patient no 1

Postoperatively the child initially did well but after a few hours he developed very low P_{O_2} values in spite of ventilator treatment and died the same evening

Autopsy Candida endocarditis was found in the right ventricle with involvement and ulceration of both the septal and the anterior tricuspid leaflets while the posterior leaflet was normal The left side of the heart was

Table I *Echocardiographic data obtained at serial examinations in case I*

AM amplitude = amplitude of opening of the echo from the anterior mitral leaflet AM thickness = thickness of the echo from the anterior mitral leaflet LAD/AOD = relation between left atrial dimension (LAD) and aortic root diameter (AOD) LVID = left ventricular internal dimension measured at end-diastole RVD = right ventricular dimension

	AM amplitude (mm)	AM thickness (mm)	Left atrial size (LAD/AOD)	Left ventricular size ($LVID_d$ mm)	Right ventricular size (RVD mm)
Day 1	11	4	1 4	0	1
Day 4	11	4	1 7	1	10
Day 7	10	6	1 45	3	19
Day 9	9	6	1 9	4	16
Day 11	8	7	1 9	8	16
Normal values according to the patient's weight	>6	≤	0 8–1	13–21	4–10

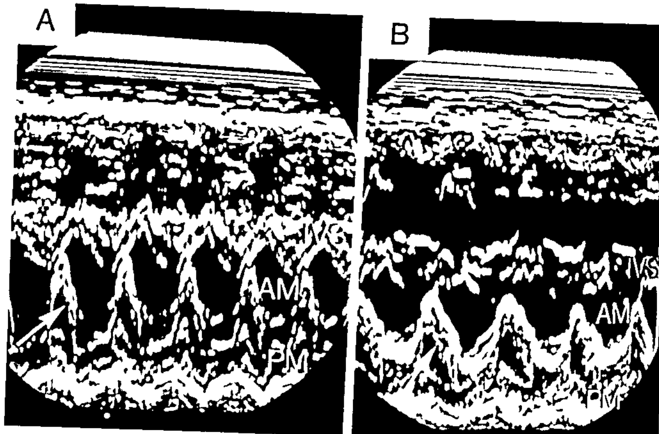


Fig 1 Echocardiogram obtained from patient no 1 on day 1 (*A*) and day 7 (*B*) with the transducer directed towards the mitral valve The anterior structures are at the top of the recording The ultrasonic beam passes the right ventricle the interventricular septum (*IVS*) and the left ventricle In the left ventricle the echoes from the anterior (*AM*) and the posterior (*PM*) leaflets are seen The echo from the anterior mitral leaflet (*arrow*) is broader than the echo from the posterior mitral leaflet presumably due to the attached vegetation On day 7 (*B*) the echo is still broader than on day 1 (*A*)

later (Day 4) gave essentially the same results On day 7 the thickness of the echo from anterior mitral leaflet had increased further (Fig 1B) and the size of the left and right ventricle had also increased (Table 1) At the last two examinations (Day 9 and 11) the amplitude of movement of the echo from the anterior mitral leaflet had decreased slightly The thickness of the echo from the anterior mitral leaflet had increased further The echo from the posterior mitral leaflet was still normal at the last examination The size of the left atrium and left ventricle had increased further (Table 1)

In summary these echocardiographic examinations showed a gradual increase in thickness of the anterior mitral leaflet a somewhat reduced amplitude of movement of the anterior mitral leaflet and a normal echo from the posterior mitral leaflet A gradual increase in size of the left atrium the left ventricle and the right ventricle could also be demonstrated

These findings could be explained by a bacterial endocarditis with a vegetation on the anterior mitral leaflet a severe mitral regurgitation and congestive heart failure

Case 2

A newborn boy was admitted to the department of pediatric surgery soon after birth because of small urinary volumes and bladder dilatation A urethral valve was diagnosed and the child was operated on at the age of five days with bilateral ureterostomy Postoperatively there was local infection with candida albicans which was treated with oral cotrimoxazol One week postoperatively the patient developed signs of urosepsis Urinary cultures grew Klebsiella and the child was treated with antibiotics and improved At the age of two months the urethral valve was resected and because of urosepsis he was treated with various antibiotics During the following months his general status deteriorated and several cultures from urine blood skin and exudate from the right knee grew candida albicans Cystoscopy revealed white masses in the bladder representing candida and ophthalmological examination revealed changes in the vitreous body which were suspected to be of candida origin Cardiac examination at the age of 3 5 months revealed a systolic murmur close to the sternal border with maximal intensity in the fourth intercostal space A chest X ray showed moderate cardiomegaly The electrocardiogram showed signs of right ventricular hypertrophy and right atrial dilatation Two-dimensional echocardiography revealed a mass in the right ventricle which was interpreted as probably due to candida endocarditis (Fig 2) The child developed signs of congestive heart failure He was given intensive antimycotic therapy digitalis and diuretics

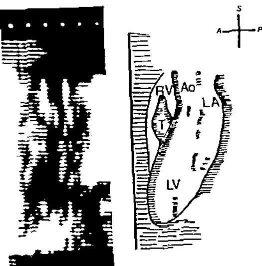


Fig 2 Two-dimensional echocardiogram (still frame from cine film) obtained with the transducer along the long axis of the heart together with schem tic drawing showing the tumor mass (*T*) in the right ventricle (*RV*) *Ao*= aortic root *LA*=left atrium *LV*=left ventricle *A*= anterior *P*=posterior *S*=superior *I*=inferior

During three weeks of treatment the boy first im proved a little but then slowly deteriorated Cultures from blood and urine were negative during treatment but since the child did not improve and the echocardiographic examinations still showed the same large mass in the right entricle surgery was considered necessary At operation using cardio-pulmonary bypass the right atrium was first opened and a large vegetation was found extending from the base of the septal tricuspid leaflet which was partially destroyed As much as possible of the mass was removed through the right atrium and the right ventricle was then opened so that the main part of the mass could be reached and extirpated (Fig 3)

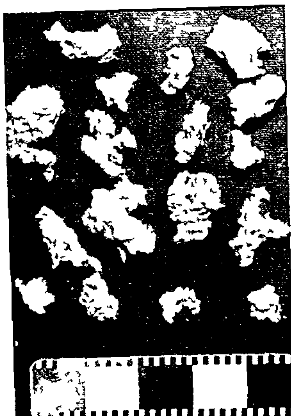


Fig 3 Photograph of the candida vegetations removed from the right ventricle during operation on patient no ?

Postoperatively the child initially did well but after a few hours he developed very low P_0 values in spite of ventilator treatment and died the same evening

Autopsy Candida endocarditis was found in the right ventricle with involvement and ulceration of both the septal and the anterior tricuspid leaflets while the posterior leaflet was normal The left side of the heart was

Table I *Echocardiographic data obtained at serial examinations in case 1*

AM amplitude = amplitude of opening of the echo from the anterior mitral leaflet AM thickness = thickness of the echo from the anterior mitral leaflet LAD/AOD = relation between left atrial dimension (LAD) and aortic root diameter (AOD) LVID = left ventricular internal dimension measured at end-diastole RVD = right ventricular dimension

	AM amplitude (mm)	AM thickness (mm)	Left atrial size (LAD/AOD)	Left ventricular size ($LVID_d$ mm)	Right ventricular size (RVD mm)
Day 1	11	4	1 4	0	1?
Day 4	11	4	1 2	21	10
Day 7	10	6	1 45	3	19
Day 9	9	6	1 9	4	16
Day 11	8	7	1 9	8	16
Normal value according to the patient's weight	>6	≤	0 8–1	13–?1	4–10

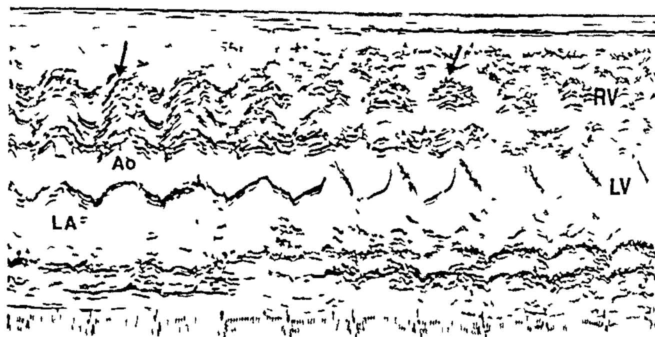


Fig 4 Echocardiogram from patient no 2 showing an M mode scan along the long axis of the heart Arrows indicate the dense mass of echoes found in the right ventricle (*RV*) and the right ventricular outflow tract anterior to the aortic root (*Ao*) *LA*=left atrium *LV* left ventricle

not involved In the main pulmonary artery an adherent candida mass was found attached to the bifurcation almost completely occluding both the left and the right pulmonary arteries The autopsy also showed bilateral hydronephrosis and a candida abscess in the left kidney

Echocardiography The examinations in case 2 were performed with an Echocardiovisor ultrasonoscope (Organon Teknika) with a fiber optic recorder using a 4 5 MHz unfocused transducer for the single element studies and a 4 5 MHz multi element transducer for the two-dimensional studies Two-dimensional studies were also performed using an ADR ultrasound real time scanner with a 3 5 MHz transducer The first examination was performed when the child had developed signs of cardiac involvement at the age of 3 5 months On the two dimensional image a large echo-producing structure could be seen in the right ventricle (Fig 2) With multi element and single element technique the structure could be followed from the right ventricle to the level of the tricuspid valve into the right ventricular outflow tract where it had a width of approximately 8 mm (Figs 4 5) On the cross sectional studies the structure appeared to be attached to the base of the septal leaflet and to move into the outflow tract with each heart beat The single element recordings of the anterior tricuspid leaflet showed multiple echoes during systole while the tip of the leaflet seemed to move freely during diastole The right atrium appeared to be enlarged while the echocardiographic examinations revealed no abnormalities in the left side of the heart

The child was followed with repeated examinations up to the time of operation but no decrease in the size of the mass could be shown in spite of the intensive antibiotic treatment

DISCUSSION

The echocardiographic appearance of th mitral valve vegetation in case no 1 is simila to that previously described (4 6 8 12 14) The characteristic feature is a non uniform thickening of the leaflet with a fuzzy shagg appearance of the thickening Since th thickening often appears on only a part of th valve it is important that the valves ar examined from different directions by makin careful M mode scans during continuous re cording Unfortunately we did not have acces to a fiber optic recorder allowing a continuou recording at that time To avoid a false posi tive diagnosis it is also important to use a proper gain setting of the equipment If th gain settings are too high an unusually thicl echo can be obtained from a normal leaflet Such a false positive diagnosis can be avoidec by comparing the echoes obtained with other echoes from the same patient and with the same gain settings In our patient normal echoes were obtained from the posterior mitral leaflet aortic leaflets etc

Possible sources of confusion with respect

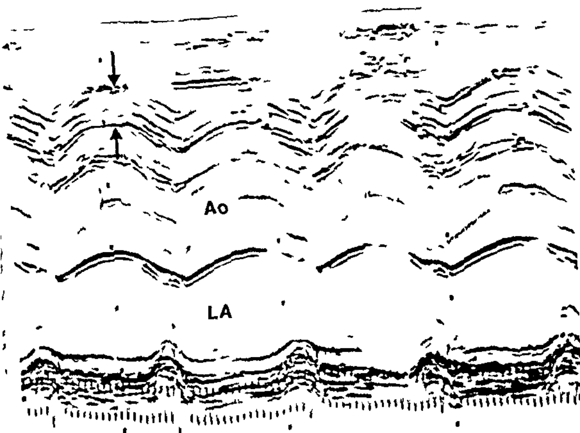


Fig. 3. Echocardiogram from patient no. 2 obtained with the transducer directed toward the left atrium (*LA*), the aortic root (*Ao*) and the right ventricular outflow tract anterior to the aorta. In the outflow tract an echo producing structure can be seen (*arrows*) representing the fungal mass.

to these echoes have been discussed by Dillon et al. (4) and Martinez et al. (14). Calcification of the valves gives a different appearance of the echoes and is unlikely in the pediatric age group. An atrial myxoma can give echoes in the mitral region with some resemblance of the described fuzzy echoes. The myxoma can be detected in the left atrium as well as related to the mitral valve. A differential diagnosis between atrial myxoma and a vegetation on the mitral valve by echocardiography appears possible as described by Dillon et al. (4).

In the pediatric age group myxomatous deposits can occur on the mitral or tricuspid leaflets in combination with other congenital heart malformations (9, 16). These deposits are often small but it is quite possible that they can cause echoes similar to those obtained from bacterial vegetations. The echocardiographic findings must therefore be evaluated together with other relevant findings.

Echocardiographic features of tricuspid endocarditis (11, 12) are similar to those reported for mitral endocarditis. In our case 2 a large echo-producing mass was found in the right ventricle during the whole cardiac cycle. Two-dimensional echocardiography was very useful in determining the exact localization of the fungal mass. Right atrial myxomas can give echoes in the same region, but then usually only during diastole (5, 7). Right ventricular tumors are extremely rare but can give the same echocardiographic appearance as in our case (1, 2, 3, 15). In our case it was not

possible to differentiate between a tumor or another echo-producing structure by echocardiography but the clinical situation made a fungal mass appear very likely

These case reports underline the value of serial echocardiographic examinations In the first case the echocardiographic data show the increasing size of the left atrium the left ventricle and the right ventricle caused by an increasing mitral regurgitation and congestive heart failure In the second case serial evaluation of tumor size was of value in monitoring the patient and in reaching a decision regarding surgical treatment

It is quite clear that valvular vegetations can be detected by echocardiography and that two dimensional echocardiography is very helpful in localizing the exact site of the vegetation What is not known however is how large the vegetation must be before it is detectable echocardiographically or how often vegetations can be detected by this method Only a systematic study of a large series of patients with endocarditis will answer these questions

REFERENCES

1 Allen H D Blieden L C Stone F M Bessinger F B Jr & Lucas R V Jr Echocardiographic demonstration of a right ventricular tumor in a neonate *J Pediatr 84* 854 1974

2 Chandraratna P A N San Pedro S Elkins R C & Grantham N Echocardiographic angiographic and surgical correlations in right ventricular myxoma simulating valvar pulmonic stenosis *Circulation 55* 619 1977

3 De Maria A N Vismara L A Miller R R Neumann A & Mason D T Unusual echocardiographic manifestations of right and left heart myxomas *Am J Med 59* 713 1975

4 Dillon J C Feigenbaum H Konecke L L Davis R H & Chang S Echocardiographic manifestations of valvular vegetations *Am Heart J 8*6 698 1973

5 Farooki Z Q Green E W & Arciniegas E Echocardiographic pattern of right atrial tum[or] motion *Br Heart J 38* 580 1976

6 Gottlieb S Khuddus S A Bolooki H & Mye[r]burg R J Echocardiographic diagnosis of end[o]carditis *Circulation 50* Suppl III 76 1974

7 Harbold N B & Gau G T Echocardiograph[ic] diagnosis of right atrial myxoma *Mayo Clin Pro[c] 48* 284 1973

8 Hirschfeld D & Schiller N B Echocardiograph[ic] localization of aortic valve vegetations *Circulation 50* Suppl III 143 1974

9 Hyams V J & Manion W C Incomplete diff[er]entiation of the cardiac valves *Am Heart J 76* 1[...] 1963

10 Keith J D Rowe R D & Vlad P *Heart disea[se] in infancy and childhood* The Macmillan Co N[ew] York 1967 2nd ed p 953

11 Kisslo J von Ramm O T Haney R Jones [...] Juk S S & Behar V S Echocardiographic eval[ua]tion of tricuspid valve endocarditis *Am J Cardi[ol] 38* 502 1976

12 Lee C C Ganguly S N Magnisalis K & Rob[...] E Detection of tricuspid valve vegetations by ec[ho]cardiography *Chest 66* 432 1974

13 Lundstrom N R Clinical applications of ec[ho]cardiography in infants and children *Acta Paedi[atr] Scand* Suppl 243 1974

14 Martinez E C Burch G E & Giles T D Ec[ho]cardiographic diagnosis of vegetative aortic bacte[rial] endocarditis *Am J Cardiol 34* 845 1974

15 Nanda N C Barold S S Gramiak R C[...] L S & Heinle R A Echocardiographic featu[res] of right ventricular outflow tumor prolapsing into pulmonary artery *Am J Cardiol 40* 272 1977

16 Persson B Johansson B W Lundstrom N[...] Mortensson W & Rausing A Cor triatriat[um] *Acta Paediatr Scand 61* 477 1972

17 Yoshikawa J Tanaka K Owaki T & Kato [...] Cord like aortic valve vegetation in bacterial en[do]carditis *Circulation 53* 911 1976

Submitted Aug 17 1978
Accepted Nov 22 1978

(N R L) Department of Paediatrics
University Hospital
S 221 85 Lund
Sweden

Acta Pædiatr Scand 68 351–355 1979

RENAL SODIUM EXCRETORY CAPACITY IN INFANTS UNDER DIFFERENT DIETARY CONDITIONS

A APERIA O BROBERGER P HERIN K THODENIUS and R ZETTERSTROM

From the Department of Paediatrics Karolinska Institutet at St Goran s Hospital and Huddinge Hospital Stockholm Sweden

ABSTRACT Aperia A Broberger O Herin P Thodenius K and Zetterstrom R (Department of Paediatrics Karolinska Institutet St Goran s Hospital Stockholm Sweden) Renal sodium excretory capacity in infants under different dietary conditions Acta Paediatr Scand 68 351 1979 —An evaluation of dietary effects on sodium (Na) homeostasis was performed in 28 healthy infants 7–13 weeks of age Each infant received during one week an ordinary formula where either the Na and/or the protein content was increased twice The high Na diets induced a significant elevation of the natriuretic response to an oral Na load The response was most pronounced in those infants receiving a high Na as well as a high protein diet The diet that was only high in protein resulted in an increased osmotic load to the kidneys but did not affect the Na excretion The maturation of renal Na excretion thus seems to be accelerated by a high Na intake and further potentiated by a high protein intake The Na excretory capacity was even after the period of a high Na diet well above the level of Na then given

KEY WORDS Infants sodium excretion renal function sodium intake

In infancy tolerance to sodium (Na) loading as well as to Na depletion is reduced (6 7) Until recently the interest has been focused on the hazards of a positive Na balance Since there is experimental evidence of an association between a positive Na balance during development and hypertension in adult life (9 11) it has been recommended that the daily Na intake should be kept low during the first year of life (1)

The recommended daily Na intake is however only an empirical value Most information on renal handling of Na has been obtained from studies in newborn pre term (3 5) and full term infants (2 5) The Na excretory capacity in infants older than one month of age has only been studied under standardized dietary conditions with a recommended i e low Na intake (4) Under such conditions the capacity to excrete Na will increase linearly during the first year of life

This study was undertaken to determine whether the tolerance to Na can be influenced by the daily Na intake during the first four months of infancy i e can two to four months old infants tolerate a higher Na intake than is generally recommended if it is given for a longer period of time

MATERIAL AND METHODS

Twenty-eight healthy infants born at term and ranging in age from 7 to 13 weeks were studied Mothers who had weaned their infants for non medical reasons were asked in the Well Baby Clinics to participate For at least four weeks prior to the study all the infants were exclusively fed an ordinary infant formula Following control determination of the Na excretory capacity extra Na and/or protein was added to the formula (Baby Semp I Semper) for one week The Na and protein contents for the three different diets are listed in Table 1 In diets where

---

This work has been supported by grants from the Swedish Medical Research Fund of the Karolinska Institute and Semper Fund for Nutritional Research

Table 1. *Sodium (Na), protein and caloric content of the three diets and of an ordinary infant formula*

	Na (mmol/l)	Protein (g/l)	Calories (kcal/l)
High Na diet	16	15	675
High Na+protein diet	16	33	670
High protein diet	7	33	680
Ordinary infant formula	7	15	675

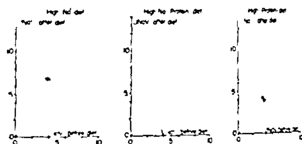


Fig. 1. Dietary effects on sodium excretion ($U_{Na}V$) in the individual infants studied.

Na and/or protein was added the content of each constituent was about twice that in ordinary formulas. In no case did the Na and/or protein enriched diets result in a change of blood pressure, gastrointestinal disturbances or irritability. The Na excretory capacity was determined again at the end of the diet period. Eight infants received the high salt diet, eleven infants received the high salt and high protein diet and nine infants received the high protein diet.

This study has been approved by the Ethical Committee of the Karolinska Institute.

Protocol for the study of the Na excretory capacity

During the study the infants were well hydrated by a diluted ordinary formula given via a stomach tube. The diluted formula was administered in an amount corresponding to 2% of body weight during the first hour and thereafter in an amount corresponding to 0.5% of body weight every half hour. About 90 min after receiving the formula, a stable diuresis was attained. A single dose of NaCl (2 mmol Na/kg) was then added to the formula. Urine was obtained by spontaneous voiding. The study was continued during a period of five to six hours after the single dose of NaCl was given. Capillary blood was taken in the middle of the total urine sampling period.

The Na concentration in urine and blood was analyzed using a flame photometer (Eppendorf). Osmolality in urine and blood was determined cryoscopically in a Knauer osmometer. Serum total protein concentration was determined with a refractometric method and serum urea with a spectrophotometric method. Erythrocyte volume fraction (hematocrit) was estimated by centrifuging the blood in heparinized glass capillaries at 10000 rpm for 5 min. Aldosterone excretion before and towards the end of the diet was determined with a radioimmunoassay method (15) in four infants who received high sodium diet only. Paired *t* test have been used in the statistical evaluation. Each infant has been its own control.

RESULTS

Some general effects of the three different diets are summarized in Table 2. The daily weight gain was higher in the infants given a high Na and high protein diet, but the difference from the other two groups was not significant. The hematocrit fell slightly but not significantly in the groups that received a high protein diet. The serum protein concentration did not change with the different diets, but the serum urea concentration increased significantly in the infants who were given high protein diets. The serum Na concentration was not affected by any of the three diets.

Table 2. *General effects of high sodium (Na) and high protein diet*

Values are mean ± standard deviation. N.S. = not significant

	Daily weight gain (g)	Hematocrit (B-EVF) (%)		S Protein (g/l)	
		Before diet	After diet	Before diet	After diet
High Na diet ($n=8$)	26.0±10.8	34.1±2.41	34.7±1.98	62.0±3.16	60.0±2.87
p value		N.S.		N.S.	
High Na+protein diet ($n=11$)	33.2±10.1	37.1±4.62	35.6±1.67	61.2±5.12	62.3±5.36
p value		N.S.		N.S.	
High protein diet ($n=9$)	30.1±10.1	42.4±11.9	40.11±9.01	65.1±4.51	64.8±3.19
p value		N.S.		N.S.	

Table 3 *Renal response to an oral sodium (Na) load during different dietary conditions*
Values are mean ± standard deviation N S = not significant

	UNa (mmol/1 73 m /hour)		Urinary flow (l/1 73 m /hour)		C_{osm} (l/1 73 m /hour)	
	Before diet	After diet	Before diet	After diet	Before diet	After diet
High Na diet (*n* = 8)	3 35±1 86	4 90±2 16	0 [illegible]±0 05	0 [illegible]6±0 05	0 07±0 04	0 09±0 0[illegible]
p value	<0 05		<0 05		N S	
High Na + protein diet (*n* = 11)	4 10±[illegible] 18	8 06±[illegible] 77	0 [illegible]6±0 04	0 27+0 08	0 09±0 06	0 19±0 05
p value	<0 001		N S		<0 01	
High protein diet (*n* = 9)	4 69±[illegible] 38	4 85±[illegible] [illegible]6	0 [illegible]0±0 06	0 [illegible]1±0 08	0 08±0 03	0 1[illegible]±0 02
p value	N S		N S		<0 05	

The capacity to excrete an oral Na load increased significantly in both groups receiving a high Na diet but in the group receiving only a high protein diet the capacity to excrete Na was unchanged after the diet The increase in Na excretory capacity was more pronounced following a high Na and high protein diet than following a high Na diet only (Fig 1 Table 3) The increase following a high Na and high protein diet was 2 fold and following a high Na diet 1 5 fold In the high protein group the relationship between Na excretion before and after the diet was 1 0 Urinary flow (Table 3) increased slightly in the group receiving only a high Na diet but not in the other two groups As expected from the increased serum urea values the osmolar clearance increased significantly in the two groups receiving high protein diets It was unchanged in the group receiving only a high Na diet Aldosterone excretion before and towards the end of the diet was determined in four infants who only received a high sodium diet The mean difference before and after the diet was not significant

[illegible] Urea (mmol/l)		S-Na (mmol/l)	
[illegible]efore [illegible]iet	After diet	Before diet	After diet
[illegible] 4 [illegible] 0 6	5 [illegible]0±3 [illegible]8	140 0[illegible]3 34	141 0±[illegible] [illegible]7
N S		N S	
[illegible]8 [illegible] 0 71	8 91±1 85	140 9[illegible] [illegible]81	140 5±3 88
<0 001		N S	
[illegible] 1 18	9 65[illegible]3 03	138 0±3 00	138 8±[illegible] 64
<0 001		N S	

DISCUSSION

The infant kidney is usually unable to rapidly excrete a given Na load When however the daily Na intake is increased for a week as in this study the capacity to excrete Na increases The results accord with the findings in previous experimental studies on puppies in which the increase in Na excretory capacity was attributed to both an increased glomerular filtration rate and to tubular rejection of Na (16)

In the present study the capacity to excrete Na was further augmented when a high protein and high Na diet was administered It is thought that a high protein diet can influence the function of the infant kidney by 1) enhancement of growth and 2) increase in osmotic load secondary to the resulting increase in serum urea concentration (10 12) A high protein diet caused a rise in serum urea concentration and hence in the osmolar clearance but did not in itself affect Na excretion This indicates that the enhancement of Na excretory capacity observed following a high Na and high protein diet was not due to transitory changes in the osmotic load It is therefore suggested that the high salt diet induces func

Table 1. *Sodium (Na), protein and caloric content of the three diets and of an ordinary infant formula*

	Na (mmol/l)	Protein (g/l)	Calories (kcal/l)
High Na diet	16	15	675
High Na+protein diet	16	33	670
High protein diet	7	33	660
Ordinary infant formula	7	15	675

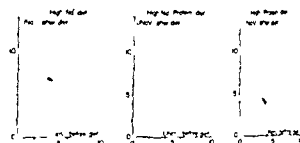


Fig. 1. Dietary effects on sodium excretion ($U_{Na}V$) in the individual infants studied.

Na and/or protein was added, the content of each constituent was about twice that in ordinary formulas. In no case did the Na and/or protein enriched diets result in a change of blood pressure, gastrointestinal disturbances or irritability. The Na excretory capacity was determined again at the end of the diet period. Eight infants received the high salt diet, eleven infants received the high salt and high protein diet and nine infants received the high protein diet.

This study has been approved by the Ethical Committee of the Karolinska Institute.

Protocol for the study of the Na excretory capacity

During the study, the infants were well hydrated by a diluted ordinary formula given via a stomach tube. The diluted formula was administered in an amount corresponding to 2% of body weight during the first hour and thereafter in an amount corresponding to 0.5% of body weight every half hour. About 90 min after receiving the formula, a stable diuresis was attained. A single dose of NaCl (2 mmol Na/kg) was then added to the formula. Urine was obtained by spontaneous voiding. The study was continued during a period of five to six hours after the single dose of NaCl was given. Capillary blood was taken in the middle of the total urine sampling period.

The Na concentration in urine and blood was analyzed using a flame photometer (Eppendorf). Osmolality in urine and blood was determined cryoscopically in a Knauer osmometer. Serum total protein concentration was determined with a refractometric method and serum urea with a spectrophotometric method. Erythrocyte volume fraction (hematocrit) was estimated by centrifuging the blood in heparinized glass capillaries at 10000 rpm for 5 min. Aldosterone excretion before and towards the end of the diet was determined with a radioimmunoassay method (15) in four infants who received high sodium diet only. Paired *t* test have been used in the statistical evaluation. Each infant has been its own control.

RESULTS

Some general effects of the three different diets are summarized in Table 2. The daily weight gain was higher in the infants given a high Na and high protein diet, but the difference from the other two groups was not significant. The hematocrit fell slightly but not significantly in the groups that received a high protein diet. The serum protein concentration did not change with the different diets, but the serum urea concentration increased significantly in the infants who were given high protein diets. The serum Na concentration was not affected by any of the three diets.

Table 2. *General effects of high sodium (Na) and high protein diet*

Values are mean ± standard deviation. N.S. = not significant

	Daily weight gain (g)	Hematocrit (B-EVF) (%): Before diet	Hematocrit (B-EVF) (%): After diet	S-Protein (g/l): Before diet	S-Protein (g/l): After diet
High Na diet (*n*=8)	26.0±10.8	34.1±2.41	34.7±1.98	62.0±3.16	60.0±[illegible].87
p value		N.S.		N.S.	
High Na+protein diet (*n*=11)	33.[illegible]±10.1	37.1±4.6[illegible]	35.6±1.67	61.2±5.12	6[illegible].3±5.36
p value		N.S.		N.S.	
High protein diet (*n*=9)	30.1±10.1	42.4±11.9	40.11±9.01	65.1±4.51	64.8±3.19
p value		N.S.		N.S.	

4 Aperia A Broberger O Thodenius K & Zetterstrom R Development of renal control of salt and fluid homeostasis during the first year of life *Acta Paediatr Scand 64* 393 1975

5 Aperia A Broberger O Thodenius K & Zetterstrom R Renal control of sodium and fluid balance in newborn infants during intravenous maintenance therapy *Acta Paediatr Scand 64* 725 1975

6 Aperia A Broberger O Thodenius K & Zetterstrom R Control of sodium homeostasis in newborn infants In *Intensive care in the newborn Symposium Sta rby Sko* Middelfart Denmark August 25–30 1975 Interscience Publ (John Wiley & Son)

7 Aperia A Broberger O Broberger U Larsson L Thodenius K & Zetterstrom R Sodium and fluid homeostasis in normal and diseased newborn In *Symposium on intensive ca e in the newborn* Durham New Hampshire USA August 8–September 1977

8 Aperia A Elinder G Herin P & Kallskog O Renal hemodynamics and filtration rate in growing rats Effect of isotonic volume expansion *27th International Congress of Physiol gy* Paris 1977

9 Bianchi G Baer P G Fox U & Guidi E The role of the kidney in the rat with genetic hypertension *Postgrad Med J 53* 123 1977

10 Calcagno P L & Lowe C U Substrate induced renal tubular maturation *J Pediatr 63* 851 1963

11 Dahl L K Salt in processed baby foods *Am J Clin Nutr 21* 787 1968

12 Edelmann C M Jr & Wolfish N M Dietary influence on renal maturation in premature infants *Pediatr Res* 2 421 1968

13 Horster M & Larsson L Mechanisms of fluid absorption during proximal tubule development *Kidney Int 10* 348 1976

14 Jose P A Slotkoff L M Lilienfield L S Calcagno P L & Eisner G M Sensitivity of neonatal renal vasculature to epinephrine *Am J Physiol 226* 796 1974

15 Sealey J E Buhler F R Laragh J H Manning E L & Brunner H R Aldosterone excretion *Circ Res 31* 367 1972

16 Steichen J J & Kleinman L I Influence of dietary sodium intake on renal maturation in unanesthetized canine puppies *Proc Soc Exp Biol Med 148* 748 1975

Submitted July 6 1978
Accepted Oct 5 1978

(P H) Department of Paediatrics
St Goran s Children s Hospital
Box 12500
S 11281 Stockholm
Sweden

Table 4 *The maximal sodium (Na) excretory capacity in relation to Na intake during different dietary conditions*

Values are mean ± standard deviation N S = not significant

	Na excretion following load (mmol/kg/6 hours)		Na intake (mmol/kg/24 hours)	
	Before diet	After diet	Before diet	During diet
High Na diet (*n*=8)	0 64±0 31	0 88±0 38	1 19±0 14	2 60±0 41
High Na protein diet (*n*=11)	0 74±0 32	1 43±0 46	1 04±0 21	2 28±0 40
High protein diet (n=9)	*0 84±0 46*	*0 83±0 45*	*1 19±0 10*	*1 13±0 10*

tional changes that will increase Na excretion and that those changes will be more pronounced if renal growth is facilitated at the same time If one considers the possibility that growth will potentiate the enhanced Na excretory capacity following the diet there are at least two possible explanations for this effect

1 Na excretion is increased due to a rise in filtered Na Glomerular filtration rate is low in the infant kidney due in part to high renal vascular resistance (8) High salt diet will result in vasodilatation and the increase in renal blood flow and glomerular filtration rate will be further augmented by a growth conditioned increase in vascular volume

2 *Na excretion is increased due to reduced fractional Na reabsorption across the paracellular pathway The water permeability of the paracellular pathway is high in the infant kidney due to leakiness of the paracellular junction (13)* The hemodynamics of the infant kidney will result in *hydrostatic pressure gradients* that favour a passive Na reabsorption across the junction (8) High Na *diet might* then change the pressure gradients across the paracellular junctions and high protein diet might alter the structure of the paracellular junctions resulting in a decreased water permeability

It has been shown in a previous study (2) that the Na excreted following the load of 2 mmol/kg most likely approximates the maximal Na excretory capacity in infants In infants who received a high Na diet the Na excretion after the salt load averaged 0 88 mmol/kg/6 hours or 3 52 mmol/kg/24 hours (Table 4) For several weeks prior to the study all the infants were given a regular formula providing a daily Na intake of about 1 2 mmol/kg while on the high Na diet they received a daily Na intake of about 2 6 mmol/kg Hence the maximal Na excretory rate after the diet considerably exceeded the intake during the high Na diet Thus the high Na diet should not result in an accumulation of Na with or without fluid The fact that the diet did not affect the serum Na concentration and hematocrit also indicates that this was not the case The present results thus suggest that the increased daily Na *intake for a period of at least a week* will contribute to the development of the Na excretory capacity *It might therefore be* an advantage to increase the daily Na intake stepwise *during the first months of life* provided that infants are healthy show no signs of renal *disease and have a negative family history* of hypertension

REFERENCES

1 American Academy of Pediatrics Committee on Nutrition Salt intake and eating patterns of infants and children in relation to blood pressure *Pediatrics 53 115 1974*

2 Aperia A Broberger O Thodenius K & Zetterstrom R Renal response to an oral sodium load in newborn full term infants *Acta Pediatr Scand 61* 670 1972

3 Aperia A Broberger O Thodenius K & Zetterstrom R Developmental study of the renal response to an oral salt load in preterm infants *Acta Paediatr Scand 63 517 1974*

4 Aperia A Broberger O Thodenius K & Zetterstrom R Development of renal control of salt and fluid homeostasis during the first year of life *Acta Paediatr Scand 64* 393 1975

5 Aperia A Broberger O Thodenius K & Zetterstrom R Renal control of sodium and fluid balance in newborn infants during intravenous maintenance therapy *Acta Paediatr Scand 64* 7 5 1975

6 Aperia A Broberger O Thodenius K & Zetterstrom R Control of sodium homeostasis in newborn infants In *Intensive care in the newborn Symposium Sta rby Slot* Middelfart Denmark August 25–30 1975 Interscience Publ (John Wiley & Son)

7 Aperia A Broberger O Broberger U Larsson L Thodenius K & Zetterstrom R Sodium and fluid homeostasis in normal and diseased newborn In *Symposium on intensive care in the newborn* Durham New Hampshire USA August 8–September 1977

8 Aperia A Elinder G Herin P & Kallskog O Renal hemodynamics and filtration rate in growing rats Effect of isotonic volume expansion *27th International Congress of Physiology* Paris 1977

9 Bianchi G Baer P G Fox U & Guidi E The role of the kidney in the rat with genetic hypertention *Postgrad Med J 53* 1 3 1977

10 Calcagno P L & Lowe C U Substrate induced renal tubular maturation *J Pediatr 63* 851 1963

11 Dahl L K Salt in processed baby foods *Am J Clin Nutr 21* 787 1968

12 Edelmann C M Jr & Wolfish N M Dietary influence on renal maturation in premature infants *Pediatr Res 2* 4 1 1968

13 Horster M & Larsson L Mechanisms of fluid absorption during proximal tubule development *Kidney Int 10* 348 1976

14 José P A Slotkoff L M Lilienfield L S Calcagno P L & Eisner G M Sensitivity of neonatal renal vasculature to epinephrine *Am J Physiol 226* 796 1974

15 Sealey J E Buhler F R Laragh J H Manning E L & Brunner H R Aldosterone excretion *Circ Res 31* 367 1972

16 Steichen J J & Kleinman L I Influence of dietary sodium intake on renal maturation in unanesthetized canine puppies *Proc Soc Exp Biol Med 148* 748 1975

Submitted July 6 1978
Accepted Oct 5 1978

(P H) Department of Paediatrics
St Goran s Children s Hospital
Box 12500
S 112 81 Stockholm
Sweden

Acta Pædiatr Scand 68 357–362 1979

SERIAL MEASUREMENTS OF THORACIC IMPEDANCE AND CARDIAC OUTPUT IN HEALTHY NEONATES AFTER NORMAL DELIVERY AND CAESAREAN SECTION

U FREYSCHUSS G NOACK and R ZETTERSTROM

F om the Departments of Clinical Physiology and of Pediatrics Karolinska Institute at Karolinska Hospital and St Goran s Hospital Stockholm Sweden

ABSTRACT Freyschuss U Noack G and Zetterstrom R (Departments of Clinical Physiology and of Pediatrics Karolinska Institute at Karolinska Hospital and St Goran s Hospital Stockholm Sweden) Serial measurements of thoracic impedance and cardiac output in healthy neonates after normal delivery and caesarean section Acta Paediatr Scand 68 357 1979 —Thoracic electrical impedance measurements were serially performed during the 1st 2nd 8th and up to the 32nd hour of life in two groups (V and S) of healthy infants In group V all 24 infants were delivered vaginally In group S all 24 infants were delivered by caesarean section for obstetrical reasons Basal thoracic impedance (Z) heart rate (HR) stroke volume (SV) and cardiac output (Q) were determined on each examination In group V Z increased from 31 9 to 34 0 ohm between 2 hours and the last recording between 8 and 32 hours SV decreased from 4 1 to 3 4 ml between 2 and 4 hours and was accompanied by a decrease of Q from 560 to 450 ml/min Heart rate slowed from 129 to 115 beats/min between 2 hours and the last recording at ≥8 hours In group S Z increased from 32 2 to 35 9 ohm between 2 and 8 hours Mean SV increased from 3 6 to 4 4 ml between 8 and 32 hours and heart rate slowed from 131 to 113 beats/min between 1 and 8 hours No significant differences were observed between the groups The accuracy of the impedance—SV and Q data cannot be validated For the most part they compare favourably with values previously obtained by soluble gas methods Serial changes may reflect not only decreasing shunts and/or increasing aeration but also changes in total fluid volume of the lungs intra or extravascular The precision of the measurements is good since reproducibility of single SV and Q determinations is higher than with standard dilution techniques The data obtained may serve as baseline values for comparison with data in infants of the same age with various anomalies

KEY WORDS Cardiac output newborns sectio thoracic impedance

Monitoring of cardiovascular function in the neonatal period has been impeded by the lack of atraumatic non invasive techniques The recent development and incrased clinical application of transthoracic electrical impedance measurements (for review see Geddes & Baker (11)) provide a simple but sensitive method for study of changes in intrathoracic air fluid ratios (3 9 19 24 29 30 34) as for example in pulmonary edema and pleural effusion (19 30 34) In infants the technique has been used in apnoea monitoring (8 12 26 27 33) Since the transthoracic impedance system was developed for measurement of cardiac stroke volume (15 16 28) much interest has been displayed in the use of this non invasive method for cardiac output determination Thus it has been used during anaesthesia surgery and in patients with myocardial infarction (10 13 18 30) In children with congenital heart disease cardiac output data obtained by impedance cardiography and by dye dilution or Fick principle (17) were found to be comparable in subjects without intracardiac shunts or valvular insufficiencies In patients with left to right shunts the impedance cardiac output values showed a good correlation with pulmonary blood flow (17)

In the neonatal period measurement of the intrathoracic air fluid ratio and stroke vol

Fig 1 Recording procedure Explanation is given in text

Three output signals were recorded on a multi-channel graphic recorder (Mingograph Siemens Elema Sweden) an ECG (taken with electrodes 1 and 4) dZ/dt and phonocardiogram As the baseline shifted with respiration only tracings at the zero line were used for calculations i e the same procedure employed in recordings of apnoea

In each subject the average of five beats was used to calculate one stroke volume Cardiac output was determined by multiplying the stroke volume by heart rate which was obtained from the ECG Each measurement represents the mean value of two or three determinations

A complete series of observations i e at 1 2 4 8 16 4 and 3 hours of life was usually not obtained as no measurements were made in the evening or at night

The level of significance between paired observations or group means is indicated as follows $p<0\ 001$ p 0 01 $p<0\ 05$ not significant=N S

RESULTS

Basal impedance (Z) stroke volume heart rate and cardiac output on each examination were analyzed and compared both within and between the two groups Mean values ±S D are given in Fig 2

In group V paired observations indicated an average increase of Z from 31 9 to 34 0 ohm ($p<0\ 05$) between the measurements at 2 hours and the time for the last recording at 8 24 or 32 hours Paired differences between other intervals were not significant The estimated stroke volume decreased from a mean of 4 1 to 3 4 ml ($p<0\ 01$) between 2 and 4 hours and the change was associated with a decrease of cardiac output from a mean of 560 ml/min to 450 ml/min ($p<0\ 05$) Both variables increased between 4 and 32 hours to 4 9 ml ($p<0\ 01$) and 557 ml/min ($p<0\ 05$) respectively Within other time intervals neither of the two variables changed significantly The mean heart rate slowed from 129 to 115 bpm ($p<0\ 01$) between 2 hours and the hour of the last recording at 8 hours or later

In group S serial observations demonstrated an increase of Z_0 from a mean of 32 2 to 35 9 ohm ($p<0\ 05$) between 2 and 8 hours no significant changes were noted within other intervals

The mean value of stroke volume increased from 3 6 to 4 4 ml ($p<0\ 05$) between 8 and 32 hours Cardiac output increased within the same interval from an average of 406 ml/min to 506 ml/min ($p<0\ 01$) Other changes in the two variables were not significant The mean heart rate slowed from 131 to 113 bpm ($p<0\ 01$) between 1 and 8 hours and did not significantly change thereafter

On comparing the mean values in *groups V and S* at comparable hours the only differences demonstrated were a somewhat greater stroke volume in group S at 4 hours ($p<0\ 05$) and a higher Z at 8 hours within the same group ($p<0\ 01$)

The reproducibility of a single Z_0 determination using the formula $c=SD_{diff}\ 100/\sqrt{2}\ x$

Fig 2 Serial changes in basal impedance (Z) stroke volume cardiac output and heart rate in group V (● ● vaginal delivery) and group S (■ ■ caesarean section) Paired mean differences ±S D are given

Table 1 *Vital characteristics of healthy newborns delivered normally (V) and by caesarean section (S)*

Mean values ±S D and significance (sig) of mean value differences are given

	V	Sig	S
Number	24		24
Weight g	3 520±454	NS	3 327±534
Length cm	51±1 8	*	49±1 7
Apgar score			
1 min			9 0±0 6
5 min			10 0±0 0
Distance between electrodes 2 and 3 cm	7 1±0 6	NS	7 3±0 6
Circumference electrode 3 cm	34 4±1 5	NS	33 4±2 2
Mother's age yr	27 9±4 7	NS	28 6±4 9

ume–cardiac output should be of clinical value in the assessment of various conditions associated with respiratory distress No data on such stroke volume measurements in healthy term infants have however been found in a search of the literature In a recent report Godfrey and co workers (6) presented cardiac output measurements obtained with impedance cardiography in 32 pre term infants of 2–81 days of age The data are of limited value for reference purposes however because of the wide variations in gestational and actual age Serial measurements are particularly needed for the first day of life when shunts of varying direction may be present For this reason the aim of this study was to obtain serial observations on the intrathoracic air fluid ratio as reflected by basal impedance and on the cardiac output during the first day of life in healthy newborns A comparison will also be made with a series of healthy infants delivered by caesarean section with a view to observing changes in the relation pulmonary air/fluid during adaptation to extrauterine life Finally it is hoped that the data acquired shall provide baseline values for comparison with those obtained from a current investigation of subjects with various conditions of the same age The value of monitoring transthoracic impedance in infants with respiratory difficulty for an early diagnosis of pneumothorax has already been reported (22)

MATERIAL

Group V (Table 1) consisted of 24 healthy infants (7 girls 17 boys) who were born at term to healthy mothers after spontaneous uncomplicated vaginal deliveries Group S (Table 1) consisted of 24 healthy infants (15 girls 9 boys) who were delivered by caesarean section at an average of 39 weeks of gestational age Only healthy mothers were included in the study and the indications for section were obstetric i e abnormal pelvis (n=14) placenta praevia (n=1) breech and crown presentations (n=2) movable foetal head (n=1) elderly primiparae or prolonged delivery (n=4) uteral fistula (n=1) and suspected placental insufficiency (n=1) In both groups the infants were in good condition and the perinatal course was uneventful The umbilical cord was clamped within about one minute at the vaginal deliveries and usually within 30–45 sec at caesarean sections In group S capillary hematocrits were obtained in 12 of the subjects within two hours after birth Ordinary values were measured (mean 59±8 8%)

METHOD

Thoracic impedance data were obtained using four aluminized Mylar tape electrodes (3 M Co Minnesota) attached around the patient s chest (see Fig 1) and an impedance cardiograph (IFM Minnesota Model 304 A) A constant sinusoidal alternating current of 4 mA at a frequency of 100 kHz was applied to the outer electrodes 1 and 4 and from the two inner electrodes 2 and 3 the standing impedance i e basal impedance (Z) was read on a digital display Both potential and current electrodes are internally connected to isolation transformers so that the maximal possible earth leakage current to the patient is limited to 40 μA A crystal microphone was placed over the second left intercostal space in order to record a phonocardiogram thus facilitating proper timing of the 2nd heart sound The electrodes which are radiolucent to X ray were kept in place during all of the recordings Stroke volume was calculated using a modification of Kubicek and coworkers method (15) with the formula

$$\text{Stroke volume} = P\ (L/Z\ )^2\ T\ dZ/dt$$

where P=hematocrit-dependent resistivity of blood (ohm cm) (15) L=distance (cm) between electrodes 2 and 3 Z_0=basal impedance (ohm) T=ventricular ejection time (sec) and dZ/dt (ohm/sec)=the first derivative of the diminution of Z_0 occurring during the rapid ejection phase of the cardiac cycle According to this formula the region between electrodes 2 and 3 is regarded as a cylindrical homogenous conductor in which there is a proportional change in basal impedance with the ejection of each stroke volume A value of 257 ohm cm was used for resistivity of blood since it corresponds to a hematocrit of 60% i e the average hematocrit in infants (23) This value was not significantly different from that measured in group S

tions in hemodynamic data with time after birth

In the present study the accuracy of the stroke volume–cardiac output data cannot be validated In comparison to the few available cardiac output determinations in healthy in fants (1) the values obtained by the imped ance technique are about 30% lower but of higher reproducibility Our results (165 to 159 ml/min body weight) obtained from the 1st to the 32nd hour compare favourably with esti mations of *Q*pc eff (4 5) and cardiac output (31) in healthy infants of the same gestational age and during the first day of life The values are lower than those obtained by Costeloe et al in pre term infants (mean 205 ml/kg BW) which may be related both to the different re sistivity factor used and to the significantly lower hematocrit values in the latter study where the majority were less than 50%

Serial cardiac output measurements such as those reported here have not been found in the literature where repeated observations usually represent grouped mean values obtained at dif ferent ages during the first days of life (4 7) the only consistent changes with time in both groups that were found in this study were a slowing of the heart rate and an increase in the air fluid ratio (Z) Both variables may indicate a decreasing left to right shunt and/or in the case of *Z* an increasing aeration of the lungs In conclusion the chief value of impedance cardiography as an investigative method in neonates is probably its ability to monitor and detect deviations from the normal sequence of cardiopulmonary adaptation

ACKNOWLEDGEMENTS

This study was supported by a grant from the Swedish Medical Research Council (K75 9X-4677-01)

REFERENCES

1 Arcilla R A Willam O Wallgren G Hanson J S Gesner I H & Lind J Quantitative studies of the human neonatal circulation *Acta Paediatr Scand* Suppl 179 196 pp 3-4
Baker L E Judy W V Geddes L E Langley F M & Hill D W The measurement of cardiac output by means of electrical impedance *Cardiovasc Res Cent Bull* 9 135 1971

3 Berman I R Scheetz W L Jenkins E B & Hufnagel H V Transthoracic electrical impedance as a guide to intravascular overload *Arch Surg* 10? 61 1971

4 Brady J P & Rigatto H Pulmonary capillary flow in the newborn infant A new method using the ple thysmograph and nitrous oxide *Pediatrics* 48 ?07 1971

5 Chu J Clements J A Cotton E K Klaus M H Sweet A Y & Tooley W H Neonatal pul monary ischemia *Pediatrics* 40 709 1967

6 Costeloe K Stocks J Godfrey S Mohapatra S N & Hill D W Cardiac output in the neonatal period using impedance cardiography *Pediatr Res* 11 1171 1977

7 Cotton E K Cogswell J J & Cropp G J A Measurements of effective pulmonary blood flow in the normal newborn human infant *Pediatrics* 47 5 0 1971

8 Daily W J R Klaus M & Meyer H B P Apnea in premature infants monitoring incidence heart rate changes and an effect of environmental temperature *Pediatrics* 43 510 1969

9 Denniston J C & Baker L E Measurement of pleural effusion by electrical impedance *J Appl Physiol* 38 851 1975

10 Gabriel S Atterhog J H Oro L & Ekelund L G Measurement of cardiac output by impedance cardiography in patients with myocardial infarction *Scand J Clin Lab Invest* 36 29 1976

11 Geddes L A & Baker L E *Principles of applied biomedical instrumentation* Wiley New York 1968

1? Hamilton L H & Burns W T Impedance measur ing ventilation monitor for infants *Ann Biomed Eng* 1 3 4 1973

13 Hill D W & Lowe H J The use of the electrical impedance technique for the monitoring of cardiac output and limb bloodflow during anaesthesia *Med Biol Eng* 11 534 1973

14 Hill D W & Thompson F D The effect of haema tocrit on the resistivity of human blood at 37°C and 100 kHz *Med Biol Eng* 13 18 1975

15 Kubicek W G Karnegis J N Patterson R P Witsoe D A & Mattson R H Development and evaluation of an impedance cardiac output system *Aerospace Med* 37 1 08 1966

16 Kubicek W G Patterson R P & Witsoe D A Impedance cardiography as a noninvasive method to monitor cardiac function and other parameters of the cardiovascular system International conference on bioelectrical impedance *Ann NY Acad Sci* 170 7-4 1970

17 Lababidi Z Ehmke D A Durnin R E Leaver ton P E & Lauer R M Evaluation of impedance cardiac output in children *Pediatrics* 47 870 1971

18 Lenz R J Thomas T A & Wilkins D G Car diovascular changes during laparoscopy Studies of stroke volume and cardiac output using impedance cardiography *Anaesthesia* 31 4 1976

averaged 2.8% ($p<0.05$) Corresponding values for stroke volume and cardiac output were 9.9 ($p<0.05$) and 10.3 ($p<0.05$)% respectively

DISCUSSION

Non invasive measurements of cardiac output in infants have hitherto relied upon methods determining the rate of uptake of an inert soluble gas e g freon (5) or nitrous oxide (N_2O) (4 6 7 25) As blood bypassing ventilated portions of the lung cannot be measured the methods are unreliable in patients with shunts or with uneven ventilation in relation to perfusion The flow measured is therefore called effective pulmonary blood flow ($\dot{Q}$pc eff) The distinction between $\dot{Q}$pc eff and true pulmonary blood flow should be kept in mind

Impedance cardiography offers an even more atraumatic rapid and easy technique for the determination of pulmonary blood flow Experimental and clinical investigations have demonstrated good reproducibility being usually better than for invasive standard dilution techniques (2 10 15) In the presence of left to right shunts impedance cardiac outputs correlate with pulmonary blood flow rather than with systemic blood flow (17)

Recently Godfrey and co workers compared impedance cardiac output data with $\dot{Q}$pc eff measured by the N_2O rebreathing technique in 32 premature infants and found significant correlations between the methods Interest was focused on the effect of the hematocrit dependent electrical resistivity of blood on the calculated impedance stroke volume With the resistivity factor (20) they used cardiac output determined by the impedance technique was significantly higher than the corresponding values obtained with the rebreathing technique in infants with hematocrits above 35% Using Kubicek s data however, the two cardiac output methods correlated fairly well A comparison of cardiac output data in anemic adult patients using the same impedance technique and a radioisotope technique also revealed a good correlation $r=0.86$ (14)

From what was said earlier it is obvious that the rebreathing technique is not ideal as a reference method because of its inability to measure shunted blood Left to right shunts may possibly be detected by recirculation of the tracer gas while neither right to-left shunts nor abnormalities of ventilation and perfusion can be recognized Shunts cannot be differentiated by the impedance technique either since the short circuited flow will be included in the cardiac output

In the present study the reduction of cardiac output in normally delivered infants between 2 and 4 hours of life may be related to the disappearance of shunting previously demonstrated by other authors with oximetry (21 32) and dye dilution (1) The rise of the intra thoracic air fluid ratio i e Z_0 between 2 hours and the last recording are in accordance with the increase of Z_0 from 2 to 12 hours of life that was observed by Olsson & Victorin (24) Although the absolute values are not comparable because of differences in technique and instrumentation the changes are relevant

Olsson & Victorin have reported a more rapid rise of the transthoracic impedance in vaginally delivered infants with late clamping of the umbilical cord and hence a larger blood volume than in early clamped children The observations in group S of a more rapid sequence of Z_0 changes and of a larger average stroke volume at 4 hours after birth than the corresponding mean value in group V can be attributed to a common hemodynamic etiology viz a larger blood volume

The very few differences between groups V and S mean values (variance being similar) at the same times of measurement are striking On the other hand in view of the uneventful perinatal course there is no reason to believe that the measured cardiovascular variables should differ greatly from the healthy control material Thus the difference between the groups should rather be the sequence of varia

SCREENING OF CORD BLOOD LOW DENSITY LIPOPROTEIN CHOLESTEROL IN THE DIAGNOSIS OF FAMILIAL HYPERCHOLESTEROLAEMIA A STUDY OF 2000 INFANTS

T J C BOULTON I H CRAIG and G HILL

From the Departments of Paediatrics and Medicine the University of Adelaide the Adelaide Children s Hospital and the Royal Adelaide Hospital Adelaide South Australia Australia

ABSTRACT Boulton T J C Craig I H and Hill G (Adelaide Children s Hospital Royal Adelaide Hospital and Departments of Paediatrics and Medicine *University of Adelaide* Adelaide Australia) Screening of cord blood low-density lipoprotein cholesterol in the diagnosis of familial hypercholesterolaemia A study of 2000 infants Acta Paediatr Scand 68 363 1979 —A prospective follow up study of infants selected by cord blood total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) levels from 2000 consecutive live births was undertaken to reassess the role of cord blood screening in the diagnosis of familial hypercholesterolaemia (FH) Mean values for serum cholesterol were (mmol/l±S D) TC 1 83±0 56 LDL-C 0 90±0 49 HDL-C 0 70±0 33 TG 0 38±0 16 Seventy three of 117 infants who had had a cord TC and/or LDL-C >9 th percentile and 373 control group children (cord TC and/or LDL-C <95th percentile) were followed up at age 3–12 months Six of the 117 were hypercholesterolaemic (HC) and one child had an HC *parent positive* detection rate ≥0 05% false positive rate ≥3 7% Four control-group children were HC and had an HC parent false negative rate ≥1 1% With the possible exception of detecting FH in a child with a known affected parent cord blood screening appears to be unreliable *for the diagnosis of FH*

KEY WORDS Cord blood screening cord serum low-density lipoprotein cholesterol familial hypercholesterolaemia

An elevated serum cholesterol may be due to familial *hypercholesterolaemia (FH)* which is one of *the commonest* inborn errors of metabolism (11) The ability to detect this condition in early life may allow long term prophylactic measures to be taken *in* one group of people with an increased risk of premature ischaemic heart disease (18) Although the excess risk in FH may be partly *independent* of the actual level of serum cholesterol (28) early intervention to reduce an elevated serum cholesterol level is becoming therapeutically acceptable (19 32)

The identification of newborn *infants with* FH by *cord blood* lipid analysis has been attempted by several groups of investigators but with conflicting results (2 10 15 16 17 20 27 31) The proportions of false positive results varied considerably in these studies and the validity of cord serum LDL C as a marker for future HC remains unproven

Tsang (32) *suggested* that a Prospective follow up of much larger numbers of children *selected by* cord blood low density lipoprotein cholesterol levels will be required to definitely assess the question of false negative cord blood cholesterol levels

Thus a *prospective* follow up study was *planned* in an attempt to answer three questions

First does an elevated cord serum cholesterol level predict future hypercholesterolaemia in infancy *and childhood*? Second does LDL C provide a more sensitive marker for *future hypercholesterolaemia* than TC? Third what proportion of children with normal cord serum lipid levels subsequently become *hypercholesterolaemic*?

19 Luepker R V Michael J R & Warbasse J R Transthoracic electrical impedance Quantitative evaluation of a non invasive measure of thoracic fluid volume *Am Heart J 85* 83 1973
20 Mohapatra S N Costeloe K L & Hill D W Blood resistivity and its implications for the calculation of cardiac output by the thoracic electrical impedance technique *Intensive Care Med 3* 63 1977
21 Moss A J Emmanouilides G & Duffie E R Jr Closure of the ductus arteriosus in the newborn infant *Pediatrics 32* 25 1963
22 Noack G & Freyschuss U The early detection of pneumothorax with transthoracic impedance in newborn infants *Acta Paediatr Scand 66* 677 1977
23 Oh W & Lind J Venous and capillary haematocrit in newborn infants and placental transfusion *Acta Paediatr Scand 55* 38 1966
24 Olsson T & Victorin L Transthoracic impedance with special reference to newborn infants and the ratio air to fluid in the lungs *Acta Paediatr Scand Suppl 207* 1970
25 Orme R L Featherby E A Rigatto H Cervantes F J & Brady J P Effective pulmonary blood flow in preterm infants with and without respiratory distress A simple bedside method using nitrous oxide *Pediatrics 57* 179 1973
26 Pacela A F Miller C F Daily W J R & Sivaglio F J Jr Impedance technique for monitoring the newborn infant *Digest of the 7th International Conference on Medical and Biological Engineering* 1967 p 251
27 Pallet J E & Scopes J W Recording respirations in newborn babies by measuring impedance of the chest *Med Biol Eng 3* 161 1965
28 Patterson R Kubicek W G Kinnen E Witsoe D & Noren G Development of an electrical impedance plethysmograph system to monitor cardiac output *Proceedings of the First Annual Rocky Mountain Bioengineering Symposium* 1964
29 Pomerantz M Baumgartner R Laundson J & Eiseman B Transthoracic electrical impedance for the early detection of pulmonary edema *Surgery 66* 260 1969
30 Pomerantz M Delgado F & Eiseman B Clinical evaluation of transthoracic electrical impedance as a guide to intrathoracic fluid volumes *Ann Surg 171* 686 1970
31 Prec K J & Cassels D E Dye dilution curves and cardiac output in newborn infants *Circulation 11* 789 1955
32 Rudolph A M Drorbaugh J E Auld P A M Rudolph A J Nadas A S Smith C A & Hubbell J P Studies on the circulation in the neonatal period The circulation in the respiratory distress syndrome *Pediatrics 27* 551 1961
33 Selstam U & Olsson T Monitoring with impedance plethysmography applied to newborn infants with disturbed functional adaptation *Proceedings of the First Nordic Meeting on Medical and Biological Engineering* Helsingfors 1970 p 25
34 Van De Water J M Mount B E Barela J R Schuster R & Leacock F S Monitoring the chest with impedance *Chest 64* 597 1973

Submitted Aug 3 1978
Accepted Oct 10 1978

(U F) Department of Clinical Physiology
The Seraphimer Hospital
Box 12700
112 83 Stockholm
Sweden

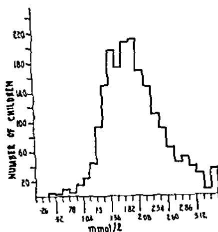


Fig 1 Frequency distribution for cord serum total cholesterol

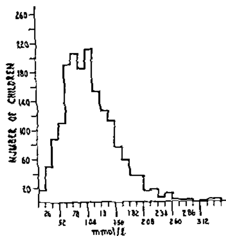


Fig 2 Frequency distribution for cord serum low density lipoprotein cholesterol

serum and at follow up for the control and HC groups are shown in Table 1 Seventy three HC neonates were followed up and 373 con trol group children were seen once or more during the first year At follow up during in fancy there were no statistically significant differences in the mean values for either TC or LDL C between any of the HC groups and the control group

HC during infancy was defined as a serum TC and/or LDL C above the age adjusted 97 5 percentile which lay at 5 9 to 6 0 mmol/l for TC from 3 to 12 months and 4 26 mmol/l for LDL C at one year The 97 5 rather than the 95th percentile was selected because it was considered most probable that children with genetically transmitted HC would fall in the higher range *310 parents of the control group* children had their serum cholesterol meas ured and from these samples the mean ± S D and 97 5 percentile (mmol/l) values for TC and LDL C were defined for fathers TC 5 17± 1 23 7 93 LDL C 3 08±0 99 4 81 for mothers TC 4 69±0 94 6 80 LDL C 2 7± 0 93 4 81 6 50 mmol/l lay at the 88th per centile for fathers and 95th percentile for mothers Of the 73 neonates who had had cord serum HC who were reviewed during

Table 1 *Values for TC and LDL C (mean ± S D mmol/l) in cord serum and at follow up of the control group children (at one year of age) and of those with cord serum HC (groups 1 to 3—see text)*

		Cord serum (x ± S D mmol/l)			Follow up value (x ± mmol/l)		
Group	Cord lipids	TC	LDL C	(n)	TC	LDL C	(n)
1	LDL C TC	95_0 17	10_0 16	3?	4 31±0 93	31±0 98	?1
	LDL C TC	3 39_0 88	31±0 57	49	4 37±1 0	2 39±1 07	28
3	LDL-C TC	3 04_0 19	1 43±0 8	36	4 36±0 75	2 59±1 27	4
4	Control	1 79_0 47	0 87_0 4	330	4 39±0 86	7 33±0 84	_7?

SAMPLE AND METHODS

The sample population comprised 2000 unselected babies born consecutively over a seven month period at the Queen Victoria Hospital Adelaide Individual details concerning the pre and peri natal experiences of the babies were not recorded on delivery for logistic reasons but were retrospectively recorded from the clinical notes of the babies who were subsequently followed up These details included the birth weight the duration of gestation the duration of labour signs of intra partum fetal distress Apgar scores at 1 and 5 min and whether resuscitation was required

The umbilical cord was routinely clamped within 1 min of delivery and 5 to 10 ml of mixed blood was taken from the placental end of the cord prior to delivery of the placenta The serum was separated and stored at 4 C within 12 hours of delivery

Biochemical analyses Cord blood high density lipoprotein (HDL C) was prepared by precipitation of other lipoproteins with heparin manganese chloride (7) The final concentration of heparin solution was 183 anti coagulability units/ml which resulted in complete precipitation of non HDL lipoproteins (5) This was confirmed by the disappearance of non HDL lipoprotein bands on polyacrylamide gel electrophoresis following heparin manganese precipitation The HDL C fraction and the second aliquot of serum were frozen at −20°C prior to lipoprotein assay with the HDL C fraction being centrifuged to remove the particulate matter which appeared on thawing Unpublished comparative studies in our laboratory have demonstrated no difference between the values for HDL C obtained on fresh serum or after freezing thawing and centrifugation of the previously precipitated sample

Serum TC and HDL C were measured using the enzymatic method of Allain (1) and the reagent manufactured by Abbott Laboratories (Diagnostic Division 820 Mission St So Pasadena Cal 91030) Analyses were performed on a Centri Chem (Union Carbide distributed by Hoffmann La Roche Basel) using 10 μl serum A reference serum supplied by the Center for Disease Control Laboratory (Georgia) was used as a primary standard

Artefactually high values for HDL C which may occur if manual enzymatic methods are used (29) are not obtained when the enzymatic method is used on a centrifugal analyser (see appendix) The value of LDL C was derived using the formula LDL C = TC−(TG/5 + HDL C) The results obtained in this way have been shown to correlate well with those obtained by ultracentrifugation (14)

Triglycerides (TG) were measured by an enzymatic method on Centrifi Chem on 10 μl serum using Calbiochem Triglyceride Glycerol Stat Pack (Calbiochem California) A reference serum with an assigned value was used as a standard The assigned value was determined using a manual enzymatic method (Calbiochem) with pure triolein as primary standard (8)

Duplicate quality control samples were included in each run to assess precision One was placed in a fixed position the other in a random position on the sample tray There was no significant difference between the results for quality control samples in fixed and random positions The means values ± standard deviations (mmol/l) and coefficients of variations (CV) for the quality control samples for TC were 1 87±0 09 (CV 5 04%) 3 19±0 09 (CV 2 9%) and for TG 0 249±0 01 (CV 4 8%) 0 59±0 02 (CV 3 6%)

Follow up studies Four groups were selected for follow up based on the cord serum levels of TC and LDL C group 1 LDL C only above the 95th percentile group 2 both TC and LDL-C above the 95th percentile group 3 TC only above the 95th percentile group 4 the control population with cord serum TC and LDL-C levels below the 95th percentile The latter group was selected in a one in four sequence from birth order from the total sample population Each mother was interviewed during her hospital stay the purpose of the study explained and her cooperation sought In the event of refusal the next child born at the hospital was selected for follow up Three hundred and thirty of the 373 babies subsequently followed had an adequate cord sample specimen collected

The children selected for follow up because of elevated serum lipid levels (groups 1 2 and 3) were reviewed between 4 and 12 months of age The children in the control population (group 4) were reviewed at the ages of 3 6 and 12 months and a non fasting blood sample taken for lipid analysis LDL C was measured at 12 months in control group children and on review in children who had cord HC The lipid levels of parents were measured by the above techniques following a 12 hour fast

Dietary and statistical analyses The average daily cholesterol and energy content of the diets were calculated from a combination of mothers recall of their babies intake over the preceding two days and a two day diet diary Calculations were made using a standard source (30) and it was confirmed by analyses of representative brands of milk and infant foods (24) Statistical analyses were done by standard methods (25)

RESULTS

Of the 2000 babies included in the sample population suitable serum specimens were obtained from 1926 One hundred and twenty nine specimens were insufficient for either HDL C and/or TG analysis so that 1797 results were obtained for LDL C The values for the mean S D and 95th percentile (in mmol/l) were for TC 1 83±0 56 2 86 for LDL C 0 90±0 49 1 82 for HDL C 0 70± 0 33 1 27 for TG 0 38±0 16 1 53 These values are similar to previous studies The frequency distribution curves for the cord serum TC and LDL C are shown in Figs 1 and 2 and the values (mean ± S D) in cord

Table 2 *Children with elevated cord serum TC LDL C and hypercholesterolaemia at follow up*

Subject	Cord TC	Cord LDL-C	3 months TC	6 months TC	1 year TC	1 year LDL C	Father TC	Father TG	Mother TC	Mother TG	Family history early CHD
L R	96	1 8_	4 78	5 70	6 40	5 75	4 81	1 81	4 13	0 95	Nil
I S	93	2 29	4 50	6 32	4 68	2 86	5 12	0 76	3 87	0 55	Nil
V B	3 56	2 13	6 71	–	6 71	3 59	–	–	4 81	0 71	No history
T F	3 75	1 95	–	9 31	5 15	3 09	5 87	0 94	5 07	0 5	Nil
S B	3 17	2 11	–	6 01	6 37	4 21	6 37	1 07	5 23	1 02	P G G Fa
S M	2 68	1 90	–	5 93	6 37	4 71	7 80	3 11	4 94	–	No history

Hypercholesterolaemic parent (TC ≥6 5 mmol/l)

50 mg/day and another sub-group with an intake close to 100 mg/day

At 3 months of age 4% of the control group babies had a low cholesterol diet and 16 5% had a moderate cholesterol diet By 6 months 59% had a low cholesterol diet 38 5% a moderate cholesterol diet and 2 5% a medium cholesterol diet For the *combined results of all the children reviewed* at one year (groups 1–4 inclusive) the proportions had changed to low cholesterol diet 40% moderate cholesterol diet 46% and medium cholesterol diet 13% No correlation was found between the serum cholesterol levels and dietary intake

The possible influences of *intra partum stress factors* on cord serum lipoproteins was investigated *in those 52* babies whose cord serum LDL C had been equal or greater than the 97 5th percentile of 1 97 mmol/l This subgroup was arbitrarily selected for retrospective analysis as it was considered that the effects of adverse perinatal events on cord serum TC and LDL C would be evident in comparison with the control group Among these 52 HC neonates the cord serum TC and LDL C was higher though not significant on *t* testing for those with a birth weight less than 2500 g a gestation period of less than 37 or greater than 42 weeks and those with a duration of labour greater than 11 hours The mean values for cord TC and LDL C were the same or lower in comparison with those neonates who had not experienced these factors in those whose labour had been induced who had had any documented sign of fetal distress whose one minute Apgar score was less than eight or who had required any resuscitative measure on delivery No differences occurred in cord serum TG for any condition studied within the sample of 52 HC babies Overall the adverse factors occurred significantly more frequently in the 52 HC neonates than in the NC control group (Table 3) $p<0\ 01$

DISCUSSION

This study was designed to test the hypotheses that an elevated cord serum LDL C might be a marker for genetically determined HC and that it might prove a more sensitive marker

Table 3 *Percentage frequency of adverse fetal and perinatal events in control group children (n=382) compared with those who had a cord serum LDL C equal to or greater than the 97.5th percentile (HC group) (n=52)*

	Control group	HC group
Birth weight <1 5 kg	3 4	34 6
Apgar 1 min <6	1 6	38 5
Apgar 5 min <8	7 8	15 3
Mother aged <16	4 7	5
Gestation <37 weeks or >41 weeks	11 5	34 6
Any sign of intrapartum fetal distress	9 2	13 5
Baby needed resuscitation	9	3 1

infancy HC was detected in six with one child (S M Table 2) having an HC parent S B s father had moderate HC (6 37 mmol/l 86th percentile) and his brother also had HC (TC 6 38 mmol/l) their paternal grandfather *having died from an infarct at 55 years* No family history of premature ischaemic heart disease was present in other families S B S M and V B had HC persisting from 3 or 6 months to 12 months The other three children had subsequently normal TC levels and normocholesterolaemic (NC) parents No information was available about V B s father or his family

Among the children in the control group ten of 354 (2 8%) were HC at 3 months of age and all but one child were NC at 6 and 12 months At 6 months 10 of 310 children (3 2%) were HC and all but one other child were NC at one year At one year 8 of 273 (2 9%) were HC and all were NC on retesting within 6 weeks Overall 27 children were HC *during infancy with four having an HC parent* (>6 5 mmol/l) None of these adults had a family history of early ischaemic heart disease but two were over weight males with mixed hypertriglyceridaemia and moderate HC

False positives and negatives The follow up of the HC neonates was incomplete because of lack of interest of the parents or the families having moved with no forwarding address given The figures for the false negative and false positive results can *therefore only present* an approximation of the true picture One (S M) of the 73 children HC as neonates was HC at follow up and had an unequivocally HC parent giving a minimal positive detection rate of 0 05% (1/1 926) The false positive rate referring to those children with cord HC and subsequent *NC was 3 7% (71/1 926)* False negative rates refer to those in whom the serum TC and/or LDL C was normal in cord blood but elevated at follow up this was 1 1% (4/373)

Because of the low case finding rate the question was also asked How many of the parents had probable FH? Fourteen of the parents tested had a TC and/or LDL C above the 97 5th percentile for sex and of these parents seven had a significant family history of premature coronary heart disease (CHD) *with a parent or grandparent having had an* infarct under 65 years of age Three of these parents had one or more such relatives affected in one generation two had had both an affected parent and grandparent and two fathers had had an infarct and had both an affected father and grandfather (the child's great grandfather) None of these parents had children who were HC at one year

Non attenders There were 114 mothers and babies in the control group who failed to attend at the 3 month and 6 month visit and whose babies had not been placed in category 1 2 or 3 A randomly selected sub-group of 30 mothers was visited and the reason for default ascertained Half the mothers either refused or had no forwarding address Six lived too far distant 4 had had their babies adopted 1 baby had died and language and transport difficulties accounted for the remainder A young mobile population with a high proportion of recent non English speaking migrants is characteristic of many suburban areas of Australia and the reasons given would also account for the failure of the parents of children in categories 1 to 3 to attend for follow up In addition dietary questionnaires were completed by the visiting *health worker and these excluded the possibility* of non attending children being given a diet qualitatively different from those children seen at follow up

Dietary analysis The mean daily dietary cholesterol intake was recorded as either a low cholesterol diet (0–100 mg cholesterol intake/day) a *moderate cholesterol* diet (101–250 mg cholesterol intake/day) or a high cholesterol diet (501–750 mg cholesterol intake/day) In retrospect the 100 mg dietary cholesterol/day division may have been *set too* high for included in that group was a subgroup of children whose intake was less than

Table 2 *Children with elevated cord serum TC LDL C and hypercholesterolaemia at follow up*

Subject	Cord TC	Cord LDL-C	*3 months* TC	*6 months* TC	*1 year* TC	*1 year* LDL-C	Father TC	Father TG	Mother TC	Mother TG	Family *history* early CHD
L R	? 96	1 8	4 78	5 ?0	6 40	5 75	4 81	1 81	4 13	0 95	Nil
I S	99	? 79	4 50	6 3?	4 68	2 86	5 1	0 76	3 8?	0 55	Nil
V B	3 56	2 13	6 71	–	6 21	3 59	–	–	4 81	0 71	No history
T F	3 ?5	1 95	–	9 31	5 15	3 09	5 8?	0 94	5 07	0 5?	Nil
S B	3 1	11	–	6 01	6 37	4 21	6 37	1 02	5 23	1 0?	P G G Fa
S M	68	1 90	–	5 93	6 37	4 ?1	7 80	3 11	4 94	–	No history

Hypercholesterolaemic parent (TC ≥6 5 mmol/l)

50 mg/day and another sub-group with an intake close to 100 *mg/day*

At 3 months of age 4% of the control group babies had a low cholesterol diet *and* 16 5% had a moderate cholesterol diet By 6 months 59% had a low' cholesterol diet 38 5% a moderate cholesterol diet and 2 5% a medium cholesterol diet For the combined results of all the children reviewed at one year (groups 1–4 inclusive) the proportions had changed to low cholesterol diet 40% moderate cholesterol diet 46% and medium cholesterol diet 13% No correlation was found between the serum cholesterol levels and dietary intake

The possible influences of *intra partum stress factors* on cord serum lipoproteins was investigated *in those 52* babies whose cord serum LDL C had been equal or greater than the *97 5th percentile of 1 97 mmol/l* This subgroup was arbitrarily selected for retrospective analysis as it was considered that the effects of adverse perinatal events on cord serum TC and LDL C *would be evident in* comparison with the control group Among these 52 HC neonates the cord serum TC and LDL C was higher though not significant on *t* testing for those with a birth weight less than 2500 g a gestation period of less than 37 or greater than 42 weeks and those with a duration of labour greater than 11 hours The mean values for cord TC and LDL C were the same or lower in comparison with those neonates who had not experienced these factors in those whose labour had been induced who had had any documented sign of fetal distress whose one minute Apgar score was less than eight or who had required any resuscitative measure on delivery No differences occurred in cord serum TG for any condition studied within the sample of 52 HC babies Overall the adverse factors occurred significantly more frequently in the 52 HC neonates than in the NC control group (Table 3) $p<0 01$

DISCUSSION

This study was designed to test the hypotheses that an elevated cord serum LDL C might be a marker for genetically determined HC and that it might prove a more sensitive marker

Table 3 *Percentage frequency of adverse fetal and perinatal events in control group children (n=382) compared with those who had a cord serum LDL C equal to or greater than the 97 5th percentile (HC group) (n=52)*

	Control group	HC *group*
Birth weight <1 5 kg	3 4	34 6
Apgar 1 min <6	1 6	38 5
Apgar 5 min <8	7 8	*15 3*
Mother aged <16	4 ?	25
Gestation <37 weeks or >41 weeks	11 5	34 6
Any sign of intrapartum fetal distress	9 2	13 5
Baby needed resuscitation	9 2	23 1

infancy HC was detected in six with one child (S M Table 2) having an HC parent S B s father had moderate HC (6 37 mmol/l 86th percentile) and his brother also had HC (TC 6 38 mmol/l) their paternal grandfather having died from an infarct at 55 years No family history of premature ischaemic heart disease was present in other families S B S M and V B had HC persisting from 3 or 6 months to 12 months The other three children had subsequently normal TC levels and normocholesterolaemic (NC) parents No information was available about V B s father or his family

Among the children in the control group ten of 354 (2 8%) were HC at 3 months of age and all but one child were NC at 6 and 12 months At 6 months 10 of 310 children (3 2%) were HC and all but one other child were NC at one year At one year 8 of 273 (2 9%) were HC and all were NC on retesting within 6 weeks Overall 27 children were HC during infancy with four having an HC parent (>6 5 mmol/l) None of these adults had a family history of early ischaemic heart disease but two were overweight males with mixed hypertriglyceridaemia and moderate HC

False positives and negatives The follow up of the HC neonates was incomplete because of lack of interest of the parents or the families having moved with no forwarding address given The figures for the false negative and false positive results can therefore only present an approximation of the true picture One (S M) of the 73 children HC as neonates was HC at follow up and had an unequivocally HC parent giving a minimal positive detection rate of 0 05% (1/1 926) The false positive rate referring to those children with cord HC and subsequent NC was 3 7% (71/1 926) False negative rates refer to those in whom the serum TC and/or LDL C was normal in cord blood but elevated at follow up this was 1 1% (4/373)

Because of the low case finding rate the question was also asked How many of the parents had probable FH? Fourteen of the parents tested had a TC and/or LDL C above the 97 5th percentile for sex and of these parents seven had a significant family history of premature coronary heart disease (CHD) with a parent or grandparent having had an infarct under 65 years of age Three of these parents had one or more such relatives affected in one generation two had had both an affected parent and grandparent and two fathers had had an infarct and had both an affected father and grandfather (the child's great grandfather) None of these parents had children who were HC at one year

Non attenders There were 114 mothers and babies in the control group who failed to attend at the 3 month and 6 month visit and whose babies had not been placed in category 1 2 or 3 A randomly selected sub-group of 30 mothers was visited and the reason for default ascertained Half the mothers either refused or had no forwarding address Six lived too far distant 4 had had their babies adopted 1 baby had died and language and transport difficulties accounted for the remainder A young mobile population with a high proportion of recent non English speaking migrants is characteristic of many suburban areas of Australia and the reasons given would also account for the failure of the parents of children in categories 1 to 3 to attend for follow up In addition dietary questionnaires were completed by the visiting health worker and these excluded the possibility of non attending children being given a diet qualitatively different from those children seen at follow up

Dietary analysis The mean daily dietary cholesterol intake was recorded as either a low cholesterol diet (0–100 mg cholesterol intake/day) a moderate cholesterol diet (101–250 mg cholesterol intake/day) or a high cholesterol diet (501–750 mg cholesterol intake/day) In retrospect the 100 mg dietary cholesterol/day division may have been set too high for included in that group was a subgroup of children whose intake was less than

Table 2 *Children with elevated cord serum TC LDL C and hypercholesterolaemia at follow up*

Subject	Cord TC	Cord LDL C	3 months TC	6 months TC	1 year TC	1 year LDL-C	Father TC	Father TG	Mother TC	Mother TG	Family history early CHD
L R	2 96	1 8	4 78	5 70	6 40	5 75	4 81	1 81	4 13	0 95	Nil
I S	99	2 29	4.50	6 37	4 68	2 86	5 17	0 76	3 82	0 55	Nil
V B	3 56	2 13	6 71	–	6 21	3 59	–	–	4 81	0 71	No history
T F	3 5	1 95	–	9 31	5 15	3 09	5 8	0 94	5 07	0 57	Nil
S B	3 17	2 11	–	6 01	6 37	4 71	6 37	1 07	5 73	1 07	P G G Fa
S M	2 68	1 90	–	5 93	6 37	4 21	7 80	3 11	4 94	–	No history

Hypercholesterolaemic parent (TC ≥6 5 mmol/l)

50 mg/day and another sub-group with an intake close to 100 mg/day

At 3 months of age 4% of the control group babies had a low cholesterol diet and 16 5% had a moderate cholesterol diet By 6 months 59% had a low cholesterol diet 38 5% a moderate cholesterol diet and 2 5% a medium cholesterol diet For the combined results of all the children reviewed at one year (groups 1–4 inclusive) the proportions had changed to low cholesterol diet 40% moderate cholesterol diet 46% and medium cholesterol diet 13% No correlation was found between the serum cholesterol levels and dietary intake

The possible influences of *intra partum stress factors* on cord serum lipoproteins was investigated in those 52 babies whose cord serum LDL C had been equal or greater than the 97 5th percentile of 1 97 mmol/l This sub group was arbitrarily selected for retrospective analysis as it was considered that the effects of adverse perinatal events on cord serum TC and LDL C would be evident in comparison with the control group Among these 52 HC neonates the cord serum TC and LDL C was higher though not significant on *t* testing for those with a birth weight less than 2500 g a gestation period of less than 37 or greater than 42 weeks and those with a duration of labour greater than 11 hours The mean values for cord TC and LDL C were the same or lower in comparison with those neonates who had not experienced these factors in those whose labour had been induced who had had any documented sign of fetal distress whose one minute Apgar score was less than eight or who had required any resuscitative measure on delivery No differences occurred in cord serum TG for any condition studied within the sample of 52 HC babies Overall the adverse factors occurred significantly more frequently in the 52 HC neonates than in the NC control group (Table 3) $p<0\ 01$

DISCUSSION

This study was designed to test the hypothesis that an elevated cord serum LDL-C might [illegible] marker for genetically determined FH [illegible] that it might prove a more sensitive [illegible]

Table 3 *Percentage frequency of [illegible] and perinatal events in control [illegible] (n=382) compared with those [illegible] serum LDL C equal to or greater [illegible] 97 5th percentile (HC [illegible]*

Birth weight <2 5 kg			
Apgar 1 min <6			
Apgar 5 min <9			
Mother aged <16			
Gestation <37 weeks or >41 week			
Any sign of [illegible] fetal distress			
Baby needed [illegible]			

for future HC than cord serum TC alone and to determine the proportion of HC infants who had had normal cord serum lipoprotein levels Particular emphasis was placed during the design of the study on ensuring a suitably large normal control population for prospective follow up a sequential analysis of LDL C in as many cord serum samples as possible and the use of cut off points for cord blood TC and LDL C derived from the 95th percentiles of the distribution curves of the actual sample

Dominantly inherited FH can be reliably diagnosed clinically only with evidence of transmission through three generations of hyper low density lipoproteinaemia and/or the presence of tendon xanthoma However because of sociological factors in the sample reported here it was only possible to obtain circumstantial evidence of three generation transmission from a history of premature coronary heart disease (CHD) and HC in parent and child

In this study no differences were found at follow up during infancy for the mean values of either TC or LDL C between those children who had been HC or NC at birth Of the six children with cord serum HC and who were HC during infancy only one (S M) had a parent with unequivocal HC Another child S B had circumstantial evidence of FH with his father and brother having moderate HC (6 37 mmol/l) and the paternal grandfather having died from an infarct at age 55 years S M s father had marked HC (7 8 mmol/l) with no history of early CHD This high rate of false positive values the minimum estimate being 3 7% may partly be explained by the influence of adverse perinatal factors which may cause an elevation in fetal serum cholesterol and TG levels (6 9 26) Although the incidence of adverse factors such as short gestation low birth weight and signs of intrapartum stress were lower than in those with a cord serum LDL C greater than the 97 5th percentile amongst the latter group there were no differences in the mean values for TC LDL C or TG in those who had or had not experienced such an event The results therefore suggest that the individual perinatal experience of a child needs to be considered when assessing his/her cord serum lipoprotein level

The results of this study also showed that not only did those children who had been HC as neonates have similar serum cholesterol values to those who had been NC as neonates when followed during infancy but that transient HC was a common finding during the first year Amongst those in the control group altogether 7 4% were HC at 3 6 or 12 months of age This tendency to revert towards a normal serum cholesterol level was also described by Darmady (10) in her study All of her 22 HC one year olds had lower levels after a two month interval None of the parents of these control group children who became HC during infancy had the characteristics of FH with only four of these parents having moderate HC with a TC between 6 25 and 6 5 mmol/l Two of these parents were young and obese fathers with mixed hypertriglyceridaemia and hypercholesterolaemia the other two parents being healthy with no family history of early CHD However there was strong circumstantial evidence of FH in the families of two NC one year olds on the basis of paternal HC and early infarct and experience of premature CHD in these generations and in a further two families on the basis of parental HC and CHD through two generations

During the first few months of life the dietary cholesterol load may be an important influence on the serum cholesterol level (12 13) with individual variation occurring between infants in the maturity of their homeostatic mechanisms (23) so that children with FH may be NC on a low cholesterol diet (31) Indirect evidence for the emergence of a more mature homeostatic state with regard to cholesterol regulation comes from unpublished data from the ongoing study of the control group of children with evidence for tracking for lipoproteins emerging during the second year and positive association then appearing

between the parents and children s lipoprotein fractions Environmental factors thus may partly obscure the genetic influences on lipoproteins at least through infancy and prevent either the level of TC or LDL C in cord blood or during the first few months of life being predictive for future HC The findings of this study are therefore in agreement with those of Darmady (10) and Potter (27) who both found cord HC unreliable as a marker for future HC and of Goldstein (16) who approached the problem from an epidemiological angle but have also shown that cord LDL C does not improve the validity of cord blood screening The authors of other prospective studies including those of Glueck (15) Tsang (32) Greten (17) and Andersen (2) have concluded that children with FH may be detected by cord screening Although we agree that this may be the case for individual families (21 22 33) and for children of parents affected with FH (20) we propose that this does not validate population cord screening programmes We also suggest that information gained from this longitudinal study of a sample of normal children has provided useful information as to the pattern and extent of individual variability of cholesterol levels during infancy and has thus partly answered the doubts concerning the possible rates of false negative and false positive values in this screening procedure

REFERENCES

1 Allain C C Poon L S Chan C S G Richmond W & Fu P C Enzymatic determination of total serum cholesterol *Clin Chem 20* 470 1974

2 Anderson G E & Friis Hansen B Neonatal diagnosis of familial type 2 hyperlipoproteinaemia *Ped atr cs 57* 214 1976

3 Anderson G E & Nielsen H G Neonatal screening for hyperlipoproteinaemia Methods for direct estimation of cord serum VLDL & LDL *Clin Ch m Acta 66* 29 19 6

4 Anderson G E & Friis-Hansen B Neonatal hypertriglyceridaemia *Acta Paediatr Scand 65* 369 1976

5 Backorick P S Wood P D Albers J J Steiner P Dempsy M Kuba K Warnick R & Karlsson L Plasma high density cholesterol concentrations determined after removal of other lipoproteins by heparin/manganese precipitation or by ultra centrifugation *Clin Chem 2?* 18 8 1976

6 Boulton T J C Perinatal stress and its effects on fetal lipoproteins *Aust NZ J Med* in press

7 Burstein M & Samaille J Sur un dosage du cholesterol lie aux α et aux β lipoproteines du serum *Clin Chim Acta 5* 609 1960

8 Chong Kit R & McLaughlin P Fully automated all enzymatic method adapted to Gem saec centrifugal analyser with use of an aqueous triolein standard *Clin Chem 20* 1454 1974

9 Cress H R Shahen R M Laffin R & Karpowicz K Cord blood hyperlipoproteinaemia and perinatal stress *Pediatr Res 11* 19 1977

10 Darmady J M Fosbrooke A S & Lloyd J K Prospective study of serum cholesterol levels during the first year of life *Br Med J 11* 685 1972

11 Fredrickson D S & Levy R I Familial hyperlipoproteinaemia *In* J B Stanbury J B Wyngarden & D S Fredrickson (eds) *The metabolic basis of inherited disease* McGraw Hill New York 1972 3rd ed p 545

12 Fredrickson D S & Breslow J L Primary hyperlipoproteinaemia in infants *Annu Rev Med 24* 315 1973

13 Friedman G & Goldberg S J Concurrent and subsequent serum cholesterols of breast fed and formula fed infants *Am J Clin Nutr 28* 4 1975

14 Glueck C J Steiner P & Leuba V Cord blood low density lipoprotein cholesterol estimation versus measurement with the preparative ultra centrifuge *J Lab Clin Med 82* 467 1973

15 Glueck C J Heckman F Schoenfield M Steiner P & Pearce W Neonatal familial type 2 hyperlipoproteinaemia cord blood cholesterol in 1800 births *Metabolism 20* 597 1971

16 Goldstein J L Albers J L Schrott H G Hazzard W R Brerman E L & Motulsky A G Plasma lipids levels and coronary heart disease on adult relative of newborns with normal and elevated cord blood lipids *Am J Hum Genet 26* 727 1974

17 Greten H Wengeler H & Wagner H Early diagnosis of familial type II hyperlipoproteinaemia *Nut Metab 15* 128 1973

18 Kannel W B Prospects for prevention of atherosclerosis in the young *Aust NZ J Med 6* 410 1976

19 Kwiterovich P O Levy R I & Fredrickson D S Early detection and treatment of type II hyperlipoproteinaemia *Circulation 62* 37 1970

20 Kwiterovich P O Levy R I & Fredrickson D S Neonatal diagnosis of familial type II hyperlipoproteinaemia *Lancet 1* 118 1973

21 Lee G B Culley G A Lawson J J Adcock L L & Knivett W Type II hyperlipoproteinaemia in mother and twins *Circulation 39* 183 1969

22 Lewis L A Brown H B & Green J G Serum cholesterol and lipoprotein levels of familial hypercholesterolaemic infants *Circulation 35* 24 1967

23 Nestel P J Poyser A & Boulton T J C Cholesterol homeostasis in infants *Proc Aust Soc Med Res 10* 55 1977

24 Nestel P J Personal communication

25 Nie N H Hull C H Jenkens J G Steinbrenner K & Bent D H *Statistical package for the social sciences* McGraw Hill New York 1975
26 Potter J M Perinatal plasma lipid concentrations *Aust NZ J Med 7* 155 1977
27 Potter J M & Nestel P J Comparison of plasma lipids at birth and in second year of life *Aust NZ J Med 6* 450 1976
28 Slack J Risks of ischaemic heart disease in familial hyperlipoproteinaemic states *Lancet II* 1380 1969
29 Steele B W Koehler D F Azar M M Blaszkowski T P Kuba K & Dempsey M E Enzymatic determinations of cholesterol in high density lipoprotein fractions prepared by a precipitation technique *Clin Chem 22* 98 1976
30 Thomas S & Corden M *Tables of composition of Australian foods* Nutr Committee N H & M R C Aust Commonwealth Dept Health 1970
31 Tsang R C Fallat R W & Glueck C J Cholesterol at birth and age 1 comparison of normal and hypercholesterolaemic neonates *Pediatrics 53* 458 1974
32 Tsang R C Glueck C J Fallat R W & Mellies M Neonatal familial hypercholesterolaemia *Am J Dis Child 129* 83 1975
33 Wolff O H Familial hyperlipidaemias *Proc Roy Soc Med 60* 1147 1967

Submitted Dec 19 1977
Accepted Nov 24 1978

(T J C B) Department of Paediatrics
The Adelaide Children s Hospital
North Adelaide
South Australia 5006
Australia

Acta Pædiatr Scand 68 371–376 1979

TRANSEPIDERMAL WATER LOSS IN NEWBORN INFANTS

II Relation to Activity and Body Temperature

K HAMMARLUND G E NILSSON P Å ÖBERG and G SEDIN

From the Department of Paediatrics University Hospital Uppsala and the Department of Biomedical Engineering University of Linköping Sweden

ABSTRACT Hammarlund K Nilsson G E Oberg P Å and Sedin G (Department of Paediatrics University Hospital Uppsala and Department of Biomedical Engineering University of Linkoping Sweden) Transepidermal water loss in newborn infants II Relation to activity and body temperature Acta Paediatr Scand 68 371 1979 —Using a method described in a previous article the transepidermal water loss (TEWL) was studied in 10 healthy newborn infants at rest and during activity On the average TEWL was 37% higher during activity than during rest although no sweating was observed In 9 infants placed in incubators with an ambient temperature slightly above the thermoneutral range measurements were made as the body temperature rose TEWL was almost constant until a temperature of 37 1 C was reached whereupon the water loss suddenly increased as the infant started sweating

KEY WORDS Water loss water balance newborn infants neonatal intensive care temperature regulation

The effect of activity on insensible water loss (IWL) has been studied by several authors (3 5 11) but it has been difficult to distinguish the amount of water lost from the skin from the water loss during respiration In studies of insensible water loss in relation to body temperature Hey & Katz (3) have found a marked increase in water loss when the body temperature was above 37 2 C These measurements included water lost from the respiratory tract and from skin areas with both eccrine and apocrine sweat glands In previous studies it has not been possible to discriminate between the effects of activity and increasing body temperature on IWL as a change in one often induces a change in the other

With the method and measurement procedure described earlier (2 6 7 8) the transepidermal water loss can be studied during varied activity at a constant ambient temperature and humidity and with only minor changes in body temperatrure measured as deep rectal temperature It is also possible to study the effect of rising body temperature in an ambient temperature slightly above the thermoneutral range without concomitant changes in activity

In our earlier studies (2 8 9) we have avoided the effects of physical activity and high body temperature by making all measurements with the infant at rest and with a body temperature of 37 C or below The purpose of this study was to investigate the transepidermal water loss in relation to rest and activity and in relation to changes in body temperature

SUBJECTS

Measurements of the evaporation rate (ER) were performed on 19 healthy newborn infants born after 37 to 39 weeks of gestation All infants were appropriate for gestational age The measurements were performed during the first 24 hours after delivery The infant s skin was neither washed nor wiped before the measurements In 10 infants ER was measured during rest and activity (Table 1) and in 9 infants placed in an ambient temperature slightly above the thermoneutral range (4 see Table 2) ER was measured during an increase in body temperature (Table 2)

Table 1 *Infant data and measurement conditions in the activity study*

Seven infants were delivered by Caesarean section Seven infants were male three female S D = standard deviation

Infant	Gestational age (completed weeks)	Weight at birth (kg)	Length at birth (m)	Age at start of measurement (h)	Duration of measurement (h)	$\bar{T}_{skin}$ (°C) Before activity	During activity	After activity	T_{body} (°C) Before activity	During activity	After activity
1	39	3 680	0 530	1 5	1 9	35 0	35 2	35 0	36 7	36 8	36 8
2	38	3 070	0 470	2 0	2 6	35 1	35 7	35 1	36 9	36 9	36 9
3	38	3 730	0 520	3 0	1 5	35 5	35 8	35 8	36 6	36 6	36 6
4	38	3 670	0 525	1 7	1 8	36 2	36 2	36 0	36 5	36 7	36 [illegible]
5	39	2 850	0 500	1 3	1 9	34 8	35 3	34 9	36 2	36 4	36 5
6	38	3 990	0 510	1 3	1 4	36 3	36 3	36 0	36 8	37 0	37 0
7	38	3 750	0 500	2 7	1 5	35 7	35 8	35 7	36 7	36 7	36 7
8	38	2 910	0 490	2 2	1 3	35 6	35 8	35 9	36 7	36 7	6 8
9	38	3 770	0 500	1 1	1 3	35 4	35 6	35 5	36 3	36 4	36 4
10	39	2 960	0 485	2 6	1 5	36 0	35 8	35 9	36 5	36 7	36 7
Mean	38 3	3 478	0 503	1 9	1 7	35 6	35 8	35 6	36 6	36 7	36 7
±S D	0 5	0 437	0 019	0 7	0 4	0 5	0 3	0 4	0 2	0 2	0 2

METHODS

The rate of evaporation from the skin (ER) was measured by a method based on determination of the vapour pressure gradient in the air layer close to the skin surface (6 7 8) The method which has been used earlier in studies on water loss at varying ambient humidities in full term infants (2) and in low birth weight infants (9 10) allows accurate measurements without disturbing the activity of the infant The equipment for measurement of ER also gives data on the ambient humidity (RH_{amb}) and ambient vapour Pressure ($P_{H_2O\ amb}$) The ambient air temperature (T_{amb}) skin temperature (T_{skin}) and body temperature (T_{body}) were measured with a YSI tele thermometer (43 TA and 4002 using probes 405 421 and 402 Yellow Springs Ohio USA) Recordings were made with a Watanabe recording system (Watanabe Instruments Corp Tokyo Japan)

MEASUREMENT PROCEDURE

All measurements were made with the infant in an incubator (AGA MK41 or MK241 AGA Medical Lidingö Sweden) and with an inflow of air to the incubator of 8 l/min (cf 2) The infants were naked and were placed in the lateral position The ambient relative humidity was kept at 50% The ambient temperature was measured in the centre of the incubator Body temperature was measured as deep rectal temperature and skin temperatures were recorded from the skin areas where the ER measurements were made

1 Estimation of transepidermal water loss in relation to activity

ER was measured on the chest (*a*) on an interscapular skin area (*b*) and on a buttock (*c*) The transepidermal

Table 2 *Infant data and measurement conditions in the body temperature study*

All infants (four male five female) were delivered by Caesarean section S D = standard deviation

Infant	Gestational age (completed weeks)	Weight at birth (kg)	Length at birth (m)	Age at start of measurement (h)	Duration of measurement (h)	$\bar{T}_{skin}$ (°C) range	T_{body} (°C) range	T_{amb} (°C) ±S D
1	38	3 400	0 505	1 1	2 7	35 6–36 8	36 0–37 4	36 9±0 2
2	39	3 120	0 480	0 5	3 0	35 0–36 5	35 9–37 3	35 8±0 4
3	38	3 860	0 535	0 5	2 3	35 6–36 4	36 3–37 2	36 6±0 3
4	38	3 670	0 505	0 5	3 8	35 4–36 4	36 2–37 2	36 2±0 4
5	39	2 650	0 480	0 5	2 3	35 5–36 5	36 1–37 2	35 7±0 2
6	38	3 000	0 470	0 6	4 1	34 7–36 4	35 8–37 3	35 3±0 2
7	39	3 260	0 500	0 6	1 8	34 8–35 9	36 4–37 2	35 1±0 1
8	38	4 200	0 520	0 6	2 7	34 7–36 3	36 4–37 2	35 1±0 1
9	39	3 810	0 520	0 6	2 4	34 5–36 2	36 3–37 2	35 2±0 2
Mean	38 4	3 441	0 502	0 6	2 8			35 8
±S D	0 5	0 487	0 022	0 [illegible]	0 7			0 7

...m (°C)		
...efore activity	During activity	After activity
7 8	37 8	37 8
3 4	33 5	33 4
5 0	35 3	36 0
4 0	34 0	34 0
3?	33 3	33
35 8	35 9	35 6
33 ?	33 5	33 7
33 5	33 5	33 0
33 8	33 7	33 6
34 4	34 3	34 1
3 9	34 0	33 9
3 9	1 0	1 1

water loss (TEWL g/m h) i e cutaneous water loss per unit area was calculated as described previously (2) using the equation

$$TEWL = 0\ 97\overline{ER}_{h} + 1\ 37$$

$T_{\ \ p}$ was kept as constant as possible and T_{mb} T_{ki} and T_{bod} were measured at the same times as ER (Table 1)

At the beginning of each measurement series the infant was asleep and had been calm without crying for at least 0 min When measurements had been made at all three measurement points the infant was made to cry for 10 min by intermittent gentle tactile stimulation at the end of which period measurements were made with the infant crying The procedure was terminated with measurements after a period of at least 20 min during which the infant was asleep or if awake was quiet and showed little motor activity Thus values for TEWL were obtained in each infant before during and after activity (crying)

2 Estimation of transepidermal water loss in relation to body temperature

Within 30 min of birth the infant was placed in an incubator with an internal temperature ($T_{\ mb}$) slightly above the thermoneutral range (4 see Table 2) With the infant at rest ER was measured on the chest (*a*) on an interscapular skin area (*b*) and on a buttock (*c*) and TEWL was calculated Repeated measurements were made while T_{bod} rose T_{mb} was kept as constant as possible T_{sk} and T_{bod} were recorded for each measurement point The measurements were terminated when an increase in TEWL was obtained or when the infant showed obvious signs of discomfort (tachypnoea vomiting)

TREATMENT OF DATA

Data were thus obtained for ER RH $_{mb}$ $P_{H_2O\ amb}$ T_{skin} T_{amb} and T_{bod}

After calculation of TEWL for different T_{bod} values for TEWL at equidistant temperatures (0 1°C) were obtained by linear interpolation between the actual measurement values for each individual infant The arithmetic mean of the three measured skin temperatures for each TEWL was designated $T_{sk\ \ b}$ (Tables 1 and 2) All statistically significant differences reported in the following were obtained by testing paired observations (Student s *t* test)

RESULTS

Transepidermal water loss in relation to activity

The mean TEWL before activity was 5 3±1 1 (S D) g/m²h During activity an increase to 6 4±1 8 (S D) g/m²h was observed The mean TEWL after a period of rest following activity was 3 9±0 6 (S D) g/m h (Fig 1)

Thus a mean increase in TEWL of 20±14 (S D) % was obtained when the values before and during activity were compared in each infant After the period of activity the mean TEWL decreased and values lower than those before activity were obtained (Fig 1) The mean TEWL during activity was 37±23 (S D) % higher than the mean value for the periods of rest in each infant

In the infants with a high TEWL before activity TEWL was also high during activity and in most cases also after activity

During activity the mean $T_{skin(a\ b\ c)}$ was 0 2 C higher than during the periods of rest ($p<0\ 05$) The mean T_{body} was 0 1 C higher both during and after activity than before activity ($p<0\ 01$)

Transepidermal water loss in relation to body temperature

In Fig 2 the TEWL values for each infant (left part) and the mean TEWL values (right

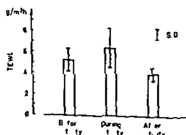


Fig 1 TEWL before during and after activity in 10 newborn infants S D =standard deviation

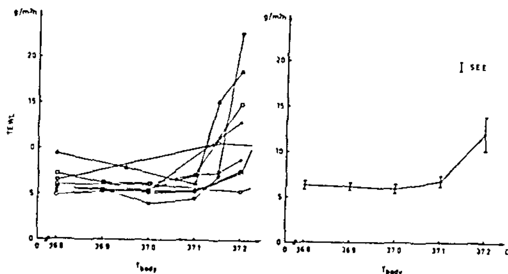


Fig. 2. TEWL in relation to body temperature in 9 newborn infants. Individual values on the left, mean values on the right. S.E.E. = standard error of estimate.

part) are given in relation to T_{body}. A rise in TEWL occurred when T_{body} was around 37.2°C. As can be seen in the right part of the figure, small S.E.E. were obtained in the T_{body} range of 36.8–37.1°C. When T_{body} rose from 37.1 to 37.2°C the mean TEWL increased by nearly 80% and scattering of the individual TEWL values occurred. The absolute TEWL values at a T_{body} higher than 37.2°C are of limited relevance according to the definition of TEWL (cf. 2 and Discussion).

DISCUSSION

In studies of transepidermal water loss in newborn infants it has been difficult to distinguish between effects of environmental factors, gestational age, body temperature and activity. The only way to obtain better knowledge of TEWL is to investigate each factor separately while the others are kept as constant as possible.

Our previous studies have been made with the infant at rest, with a normal body temperature and *in a constant environment within the* thermoneutral range (4). The air velocity in the incubator has been kept low. Measurements have been made with a 50% relative humidity except when the effects of environmental humidity have been examined. As during these studies changes in activity and body temperature have been noted to affect TEWL, a systematic study of the effect of changes in activity and body temperature has been consid-ered necessary.

In the present study TEWL was 37% highe[r] during activity than during periods of rest. This difference exceeds the increase in insen-sible water loss (IWL) reported by Hey & Katz (3). In IWL respiratory water loss is in-cluded. *The finding of Zweimüller & Preinin[ger]* (11) that IWL could increase by a factor of a[t] least 1.7 during periods of activity implies tha[t] the respiratory water loss increases greatl[y] with crying and/or motor activity. Furthe[r] comparisons with earlier studies are not val[id] as either the duration or the degree of activit[y] (3, 5) or the environmental humidity (5, 11) have not been specified exactly.

Disturbances of the sleeping newborn infan[t] cause arousal, *motor activity and crying*. It is probable that motor activity in the form o[f] movements and crying gives rise to increased heat production in the working muscles, which would lead to an increased body temperature if the thermoregulation of the infant did not intervene. The means available for cooling are *increased blood flow through the skin and ac-*tivation of the sweat glands.

In this study all infants showed a visible increase in skin perfusion during activity, while no visible sweating was observed. The small changes in skin temperature in this study can only have contributed to a minor part of the *increase in TEWL during activity by raising* the diffusion rate of water through the skin. It

therefore seems probable that a considerable part of the increase in TEWL was mediated through the sweat glands by the combined effect of changes in the local skin temperature and activation of the central thermoregulatory system (cf 1)

The fact that the TEWL was lower in the period of rest after activity than before activity may be partly explained by age related changes in TEWL in this early period of life (see Table 1) This has also been observed in some of our other studies and will be discussed further in a future article Although no differences were observed in the state of rest before and after activity a contribution of such effects cannot be excluded

In the body temperature range 36 0–37 0 C the activity of the newborn infants could be kept constant and low while the body temperature increased This was also true up to a body temperature of 37 2 C but at this point visible sweating occurred and was in most infants followed by an increase in activity flushing and an increase in the respiratory rate Thus at higher body temperatures the effects of body temperature and activity on the TEWL could not be kept separate in this study Such changes in activity with increasing body temperature have not been reported in other studies (3)

The equation for calculating TEWL was based on data obtained in the body temperature range 36 0–37 0 C and the effects that could have been caused by the uneven distribution of the apocrine sweat glands were thereby avoided The TEWL obtained at a T_{body} of 37 2 C underestimates the actual cutaneous water loss Further at this body temperature the TEWL given in Fig 2 cannot be considered as the maximum value as sweating had not reached a steady state

According to Benzinger (1) the human thermostat is situated in the hypothalamus and works with a temperature set point that controls sweating Deep rectal temperature does not allow conclusions to be drawn as to the set point in adults as the changes in rectal temperature are fairly slow Tympanic temperature and oesophageal temperature reflect the hypothalamic temperature more accurately The differences between rectal and tympanic temperatures and their relation to the induction of sweating have not come within the frame of this study In infants with high activity it was observed that sweating was obtained at a somewhat lower body temperature than in infants at rest These infants have not otherwise been included in this report or in Fig 2 and Table 2 Induction of sweating at a lower deep rectal temperature may be the result of a raised hypothalamic temperature in the more active infants

CONCLUSIONS

Under the conditions in which this study was performed it was concluded that

1 Transepidermal water loss increases considerably with activity without visible sweating or appreciable increases in deep rectal temperature

2 Transepidermal water loss at rest increases markedly with a rise in deep rectal temperature above 37 1 C

ACKNOWLEDGEMENTS

The study was supported by grants from the Swedish Medical Research Council (projects B78 19X-04998-0 B79 19X-04998-03) The authors wish to thank B Ostmark for skilful laboratory and technical assistance O Wistrand for expert technical assistance and I Grandin and C Odenyo for typing the manuscript The valuable help of B Bergman and her collaborators at the Neonatal Unit of the University Hospital Uppsala is also gratefully acknowledged

REFERENCES

1 Benzinger T H Heat regulation Homeostasis of central temperature in man *Physiol Rev* 49(4) 671 1969

Hammarlund K Nilsson G E Oberg P Å & Sedin G Transepidermal water loss in newborn infants I Relation to ambient humidity and site of measurement and estimation of total transepidermal water loss *Acta Paediatr Scand* 66 553 1977

3 Hey E N & Katz G Evaporative water loss in the newborn baby *J Physiol* 200 605 1969

4 Hey E N & Katz G The optimum thermal environment for naked babies *Arch Dis Child* 45 328 1970
5 Little J A Brodsky W A & Greathouse R The insensible weight loss of newborns and of older infants *Am J Dis Child* 90 630 1955
6 Nilsson G E Measurement of water exchange through skin *Med Biol Eng Comput* 15 209 1977
7 Nilsson G E On the measurement of evaporative water loss Methods and clinical applications *Linköping Studies in Science and Technology Dissertations No 11* and *Linköping University Medical Dissertations No 48* Linkoping 1977
8 Nilsson G Sedin G & Öberg Å A transducer for measurement of evaporation from the skin *Proc Internat Conference on Biomedical transducers* Paris Nov 3-7 1975 part II p 71
9 Sedin G Hammarlund K Nilsson G E & Oberg P Å Evaporative water loss in full term and premature newborn infants *Abstr No 47 6th Nordic Congress of Perinatal Medicine* Oulu Sept 15-17 1977
10 Sedin G Hammarlund K Nilsson G E & Öberg P Å The influence of environment activity and gestational age on evaporative water loss *Proc Annual Meeting of European Society for Paediatric Research No 51* Turku June 25-29 1978
11 Zweimuller E & Preining O The insensible water loss of the newborn infant *Acta Paediatr Scand* Suppl 205 1970

Submitted Aug 28 1978
Accepted Nov 29 1978

(G S) Department of Paediatrics
University Hospital
S 750 14 Uppsala
Sweden

Acta Pædiatr Scand 68 377–382 1979

A FOLLOW UP STUDY OF INFANTS WITH ADVERSE REACTIONS TO COW'S MILK

I Serum IgE Skin Test Reactions and RAST in Relation to Clinical Course

ANDERS DANNAEUS and S G O JOHANSSON

From the Department of Paediatrics and the Blood Centre University Hospital Uppsala Sweden

ABSTRACT Dannaeus A and Johansson S G O (Department of Pediatrics and the Blood Centre University Hospital Uppsala Sweden) A follow up study of infants with adverse reactions to cow s milk I Serum IgE skin test reactions and RAST in relation to clinical course Acta Paediatr Scand 68 377 1979 —47 infants with cow s milk sensitivity were followed for a period varying between 6 months to 4 years (mean 28 months) The age at onset of symptoms varied between 14 days to 0 months The clinical course was studied in relation to reaginic allergy by use of serum IgE skin prick test and RAST Infants with an immediate onset of symptoms from the gastrointestinal tract and the skin after cow s milk intake were discerned as a distinct entity having a high frequency of atopy in the family positive skin tests and positive RASTs to milk (71%) Cases with delayed reactions to cow s milk seldom had a positive RAST or skin test Most infants of both groups showed an increasing tolerance to milk In RAST positive infants the RAST titers increased significantly after onset of symptoms After having reached a peak the titers subclined in several cases The titers did not reflect the degree of milk sensitivity during the follow up period However infants who developed high titers seemed to develop tolerance more slowly than infants with low titers

KEY WORDS Atopic allergy infants IgE cow s milk allergy

Gastrointestinal dermal respiratory and anaphylactic reactions as well as a variety of other symptoms in infancy have been attributed to sensitivity to cow s milk (9) Lack of objective criteria for the diagnosis explains the varying incidence reported in the literature (0 3–7 5%) (1 5 8) A reaginic allergy reaction (type I reaction) has been suggested in cases with rapid onset and short duration of symptoms (11 13) and a type III or IV reaction has been suggested in cases with delayed onset and often prolonged duration of symptoms (12 16 17)

The diagnostic value of skin tests for milk antibodies belonging to different immunoglobulin classes are considered to be limited as positive tests are commonly found even in individuals tolerating milk (14 15 6) Since sensitivity to milk is usually transient a prospective study might elucidate some of the immunologic variables involved in the pathogenesis and the development of clinical tolerance

The aim of the present investigation has been to study the clinical course of cow s milk sensitivity and its relationship to reaginic allergy by use of serum IgE skin prick test and RAST

MATERIALS

The group under study consisted of 47 children 26 boys and 21 girls with cow s milk sensitivity who during 1974–77 were investigated at the Department of Paediatrics University Hospital Uppsala The diagnoses were based on the following criteria

1 Disappearance of symptoms after elimination of cow s milk from the diet

2 Reappearance of symptoms within 24 hours following reintroduction of cow s milk Repeated confirmatory challenge tests were performed in most children

The term cow s milk sensitivity is used for all adverse reactions to cow s milk while the term cow s milk allergy is restricted to reaginic allergy

In children with a clinical picture of malabsorption disaccharidase deficiencies and coeliac disease were excluded by jejunal biopsies lactose and xylose tolerance tests Only two children exhibited abnormal biopsies and in both cases the diagnosis coeliac disease was verified by

Table 1 *The degree of sensitivity to cow's milk at the end of the follow up period as compared to the sensitivity at the onset of symptoms*

Sensitivity to cow's milk	Group I $n=28$ (%)	Group II $n=19$ (%)
Unchanged	18	5
Possibly decreased	21	16
Definitely decreased	32	5
Complete tolerance	29	74

gluten provocation and repeated biopsies In addition to the gluten sensitivity both children exhibited gastrointestinal reactions to cow's milk which remained despite a normal lactose absorption and a restored mucosa on a gluten free diet

The children were divided into 2 groups according to the reaction onset time 28 children who had symptoms within one hour following cow's milk intake were assigned to Group I and 19 children with a more delayed onset of symptoms (range 1–24 hours) to Group II

The age at onset of symptoms varied from 14 days to 8 months in Group I and from 1 month to 20 months in Group II with an arithmetic mean of 3 3 and 4 9 months respectively

A family history of allergic disease was obtained in 64% in Group I and 26% in Group II 21% of the children in Group I had a double parental history of allergic disease compared to none in Group II

The mean duration of breast feeding was 3 0 months in Group I and 2 3 months in Group II When breast feeding was impossible as substitution for cow's milk a commercial soy bean formula Sobee® was used

METHODS

The follow up varied between 6 months and 4 years (mean 28 months) and 37 children were followed for more than 24 months Thirty five children came for a final examination including a blood sampling and skin prick tests with milk and soy bean preparations

The median number of blood samples per child was 3 (range 1–8) In all children the first blood samples were obtained within 6 months and in one half of the children within one month after the onset of symptoms The sensitivity to cow's milk was considered *possibly decreased* if according to parental estimates small amounts of milk in food stuffs were better tolerated than at the onset of symptoms and as *definitely decreased* if an increased amount of whole milk was tolerated

Challenge tests in children with a case history of anaphylactic reactions after cow's milk intake were performed carefully with 1 ml of dilution 10^{-8} of fresh cow's milk as the initial dose If no reaction was observed 1 ml of 10 times less diluted cow's milk was given every hour In a few extremely sensitive children challenge tests were considered too riskful to perform If there was no acute symptoms according to the case history and the skin test was negative 1 ml of undiluted cow's milk was given as initial dose In the absence of a reaction within 30 min 10 ml was given and 30 min later another 50 ml

Skin tests were performed as prick tests and the reactions were observed after 20 min A wheal size of 3×3 mm or more was considered positive (2) A reaction similar to or larger than the wheal obtained with a histamine solution (1 mg/ml) was regarded as strongly positive (+++) and intermediate reactions as (++) In children less than one year of age tests giving no wheal but an erythematous flare exceeding 5×5 mm were also considered positive (+) In children suspected to be extremely sensitive contact testing was performed with a drop of milk applied on the skin for 15 min Wheal and flare reactions were considered a positive test The parents were also asked to report delayed skin reactions appearing during 24 hours following the skin prick test

IgE concentrations were measured by a radioimmunosorbent technique PRIST (4) The IgE antibody activity was determined by RAST (10) using ^{125}I labeled anti De? The results were expressed in PRU/ml (Phadebas RAST units Pharmacia Diagnostics AB Uppsala) and concentrations greater than 0 2 PRU/ml were considered positive

Allergen preparations used for the in vitro tests were skimmed cow's milk without additives (0 1 ml per hundred paper discs) and a soy bean extract (? 0 ml per hundred discs) with 90% soy protein according to the specification The soy bean extract was prepared by dissolving 3 g of commercial soy protein (Soja protein HKC) in 30 ml 0 15 M saline The supernatant obtained after centrifugation was used Allergen preparations used for skin tests were fresh commercial whole milk commercial whole milk boiled for ten minutes and a 100 mg/ml solution of β lactoglobulin (BLG) (Miles Laboratories Ltd lot no 96022 04) The same soy bean extract as in RAST was used in vivo

Table 2 *Skin prick test with milk and soy bean preparations in 35 children attending the final examination*

Results obtained in the beginning of the investigation period are indicated within parentheses

Allergen	*Positive* Group I $n=20$ (%)	Group II $n=15$ (%)
BLG	85	7
Unboiled milk	80 (50)	7 (20)
Boiled milk	60	7
Soy bean	50	0

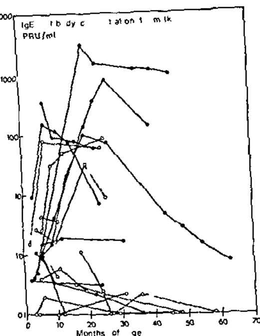


Fig. 1. IgE antibody concentrations to milk. Milk RAST positive infants with unchanged or possibly decreased sensitivity (●) and with definitely decreased sensitivity or complete tolerance (○) at the end of the follow up.

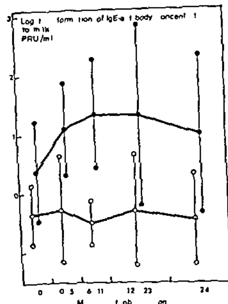


Fig. 2. IgE antibody concentrations to milk in relation to development of milk tolerance. Milk RAST positive infants with unchanged or possibly decreased sensitivity (●) and with definitely decreased sensitivity or complete tolerance (○). Geometric mean, geometric mean ±S.D.

RESULTS

In Group I one third of the children had onset of symptoms already by their first few meals of cow's milk formula. The most frequent symptoms were urticaria (71%) and diarrhoea (64%). The duration of symptoms varied from 2–24 hours. In Group II the onset of symptoms was generally more insidious and mainly confined to the gastrointestinal tract. Most children in this group developed a clinical picture of malabsorption.

Clinical course

During the follow up period several children had recurrent episodes of allergic symptoms even on a milk free diet.

In Group I asthma occurred in 25% and eczema in 43% but in Group II only one child had such symptoms. Furthermore Group I children had additional food sensitivities with reactions to egg (50%), fish (23%), pea (14%), soy bean (14%) and cereals (11%). In Group II only two children developed other food sensitivities. Soy bean formulas were initially well tolerated by all children but during the follow up four children of Group I and two children of Group II had adverse reactions to such formulas including eruption of eczema, increased itching and diarrhoea.

Five children seemed to be sensitive enough to react to the trace amounts of cow's milk protein present in breast milk when the mother ingested milk. In two cases skin prick tests with the mothers breast milk were positive but turned negative when repeated with breast milk from the same mother off cow's milk. Most children in both groups tolerated increased amounts of milk at the end of the follow up and complete tolerance was found in 29% in Group I and 74% in Group II (Table 1).

The serum IgE concentrations. At onset of symptoms significantly higher serum IgE levels were found in Group I (geometric mean 103.7 kU/l) than in Group II (geometric mean

Table 1 *The degree of sensitivity to cow's milk at the end of the follow up period as compared to the sensitivity at the onset of symptoms*

Sensitivity to cow's milk	Group I $n=28$ (%)	Group II $n=19$ (%)
Unchanged	18	5
Possibly decreased	21	16
Definitely decreased	32	5
Complete tolerance	29	74

gluten provocation and repeated biopsies In addition to the gluten sensitivity both children exhibited gastrointestinal reactions to cow's milk which remained despite a normal lactose absorption and a restored mucosa on a gluten free diet

The children were divided into 2 groups according to the reaction onset time 28 children who had symptoms within one hour following cow's milk intake were assigned to Group I and 19 children with a more delayed onset of symptoms (range 1–24 hours) to Group II

The age at onset of symptoms varied from 14 days to 8 months in Group I and from 1 month to 20 months in Group II with an arithmetic mean of 3.3 and 4.9 months respectively

A family history of allergic disease was obtained in 64% in Group I and 26% in Group II 21% of the children in Group I had a double parental history of allergic disease compared to none in Group II

The mean duration of breast feeding was 3.0 months in Group I and 2.3 months in Group II When breast feeding was impossible as substitution for cow's milk a commercial soy bean formula Sobee® was used

METHODS

The follow up varied between 6 months and 4 years (mean 28 months) and 37 children were followed for more than 24 months Thirty five children came for a final examination including a blood sampling and skin prick tests with milk and soy bean preparations

The median number of blood samples per child was 3 (range 1–8) In all children the first blood samples were obtained within 6 months and in one half of the children within one month after the onset of symptoms The sensitivity to cow's milk was considered *possibly decreased* if according to parental estimates small amounts of milk in food stuffs were better tolerated than at the onset of symptoms and as *definitely decreased* if an increased amount of whole milk was tolerated

Challenge tests in children with a case history of anaphylactic reactions after cow's milk intake were performed carefully with 1 ml of dilution 10 of fresh cow's milk as the initial dose If no reaction was observed 1 ml of 10 times less diluted cow's milk was given every hour In a few extremely sensitive children challenge tests were considered too riskful to perform If there was no acute symptoms according to the case history and the skin test was negative 1 ml of undiluted cow's milk was given as initial dose In the absence of a reaction within 30 min 10 ml was given and 30 min later another 50 ml

Skin tests were performed as prick tests and the reactions were observed after 20 min A wheal size of 3×3 mm or more was considered positive (2) A reaction similar to or larger than the wheal obtained with a histamine solution (1 mg/ml) was regarded as strongly positive (+++) and intermediate reactions as (++) In children less than one year of age tests giving no wheal but an erythematous flare exceeding 5×5 mm were also considered positive (+) In children suspected to be extremely sensitive contact testing was performed with a drop of milk applied on the skin for 15 min Wheal and flare reactions were considered a positive test The parents were also asked to report delayed skin reactions appearing during 24 hours following the skin prick test

IgE concentrations were measured by a radioimmunosorbent technique PRIST (4) The IgE antibody activity was determined by RAST (10) using ^{125}I labeled anti IgE The results were expressed in PRU/ml (Phadebas RAST units Pharmacia Diagnostics AB Uppsala) and concentrations greater than 0.2 PRU/ml were considered positive

Allergen preparations used for the in vitro tests were skimmed cow's milk without additives (0.1 ml per hundred paper discs) and a soy bean extract (2.0 ml per hundred discs) with 90% soy protein according to the specification The soy bean extract was prepared by dissolving 3 g of commercial soy protein (Soja protein HKC) in 30 ml 0.15 M saline The supernatant obtained after centrifugation was used Allergen preparations used for skin tests were fresh commercial whole milk commercial whole milk boiled for ten minutes and a 100 mg/ml solution of β lactoglobulin (BLG) (Miles Laboratories Ltd lot no 96022 04) The same soy bean extract as in RAST was used in vivo

Table 2 *Skin prick test with milk and soy bean preparations in 35 children attending the final examination*

Results obtained in the beginning of the investigation period are indicated within parentheses

Allergen	Positive: Group I $n=20$ (%)	Group II $n=15$ (%)
BLG	85	7
Unboiled milk	80 (50)	7 (20)
Boiled milk	60	7
Soy bean	50	0

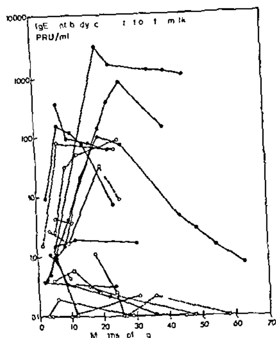


Fig 1 IgE antibody concentrations to milk Milk RAST positive infants with unchanged or possibly decreased sensitivity (●) and with definitely decreased sensitivity or complete tolerance (O) at the end of the follow up

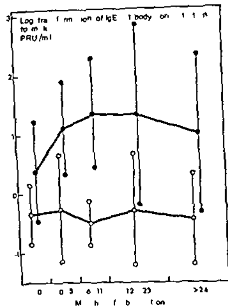


Fig 2 IgE antibody concentrations to milk in relation to development of milk tolerance Milk RAST positive infants with unchanged or possibly decreased sensitivity (●) and with definitely decreased sensitivity or complete tolerance (O) Geometric mean geometric mean ±S D

RESULTS

In Group I one third of the children had onset of symptoms already by their first few meals of cow s milk formula The most frequent symptoms were urticaria (71%) and diarrhoea (64%) The duration of symptoms varied from 2–24 hours In Group II the onset of symptoms was generally more insidious and mainly confined to the gastrointestinal tract Most children in this group developed a clinical picture of malabsorption

Clinical course

During the follow up period several children had recurrent episodes of allergic symptoms even on a milk free diet

In Group I asthma occurred in 25% and eczema in 43% but in Group II only one child had such symptoms Furthermore Group I children had additional food sensitivites with reactions to egg (50%) fish (23%) pea (14%) soy bean (14%) and cereals (11%) In Group II only two children developed other food sensitivites Soy bean formulas were initially well tolerated by all children but during the follow up four children of Group I and two children of Group II had adverse reactions to such formulas including eruption of eczema increased itching and diarrhoea

Five children seemed to be sensitive enough to react to the trace amounts of cow s milk protein present in breast milk when the mother ingested milk In two cases skin prick tests with the mothers breast milk were positive but turned negative when repeated with breast milk from the same mother off cow s milk Most children in both groups tolerated increased amounts of milk at the end of the follow up and complete tolerance was found in 29% in Group I and 74% in Group II (Table 1)

The serum IgE concentrations At onset of symptoms significantly higher serum IgE levels were found in Group I (geometric mean 103 7 kU/l) than in Group II (geometric mean

Table 1 *The degree of sensitivity to cow's milk at the end of the follow up period as compared to the sensitivity at the onset of symptoms*

Sensitivity to cow's milk	Group I $n=28$ (%)	Group II $n=19$ (%)
Unchanged	18	5
Possibly decreased	21	16
Definitely decreased	32	5
Complete tolerance	29	74

gluten provocation and repeated biopsies In addition to the gluten sensitivity both children exhibited gastrointestinal reactions to cow's milk which remained despite a normal lactose absorption and a restored mucosa on a gluten free diet

The children were divided into 2 groups according to *the reaction onset time 28 children who had symptoms* within one hour following cow's milk intake were assigned to Group I and 19 children with a more delayed onset of symptoms (range 1–24 hours) to Group II

The age at onset of symptoms varied from 14 days to 8 months in Group I and from 1 month to 20 months in Group II with an arithmetic mean of 3 3 and 4 9 months respectively

A family history of allergic disease was obtained in 64% in Group I and 26% in Group II 21% of the children in Group I had a double parental history of allergic disease compared to none in Group II

The mean duration of breast feeding was 3 0 months in Group I and 2 3 months in Group II When breast feeding was impossible as substitution for cow's milk a commercial soy bean formula Sobee® was used

METHODS

The follow up varied between 6 months and 4 years (mean 28 months) and 37 children were followed for more than 24 months Thirty five children came for a final examination including a blood sampling and skin prick tests with milk and soy bean preparations

The median number of blood samples per child was 3 (range 1–8) In all children the first blood samples were obtained within 6 months and in one half of the children within one month after the onset of symptoms The sensitivity to cow's milk was considered *possibly decreased* if according to parental estimates small amounts of milk in food stuffs were better tolerated than at the onset of symptoms and as *definitely decreased* if an increased amount of whole milk was tolerated

Challenge tests in children with a case history of anaphylactic reactions after cow's milk intake were performed carefully with 1 ml of dilution 10 [illegible] of fresh cow's milk as the initial dose If no reaction was observed 1 ml of 10 times less diluted cow's milk was given every hour In a few extremely sensitive children challenge tests were considered too riskful to perform If there was no acute symptoms according to the case history and the skin test was negative 1 ml of undiluted cow's milk was given as initial dose In the absence of a reaction within 30 min 10 ml was given and 30 min later another 50 ml

Skin tests were performed as prick tests and the reactions were observed after 20 min A wheal size of 3×3 mm or more was considered positive (2) A reaction similar to or larger than the wheal obtained with a histamine solution (1 mg/ml) was regarded as strongly positive (+++) and intermediate reactions as (++) In children less than one year of age tests giving no wheal but an erythematous flare exceeding 5×5 mm were also considered positive (+) In children suspected to be extremely sensitive contact testing was performed with a drop of milk applied on the skin for 15 min Wheal and flare reactions were considered a positive test The parents were also asked to report delayed skin reactions appearing during 24 hours following the skin prick test

IgE concentrations were measured by a radioimmunosorbent technique PRIST (4) The IgE antibody activity was determined by RAST (10) using ^{125}I labeled anti [illegible] The results were expressed in PRU/ml (Phadebas RAST units Pharmacia Diagnostics AB Uppsala) and concentrations greater than 0 2 PRU/ml were considered positive

Allergen preparations used for the in vitro tests were skimmed cow's milk without additives (0 1 ml per hundred paper discs) and a soy bean extract (2 0 ml per hundred discs) with 90% soy protein according to the specification The soy bean extract was prepared by dissolving 3 g of commercial soy protein (Soja protein HKC) in 30 ml 0 15 M saline The supernatant obtained after centrifugation was used Allergen preparations used for skin tests were fresh commercial whole milk commercial whole milk boiled for ten minutes and a 100 mg/ml solution of β lactoglobulin (BLG) (Miles Laboratories Ltd lot no 96022 04) The same soy bean extract as in RAST was used in vivo

Table 2 *Skin prick test with milk and soy bean preparations in 35 children attending the final examination*

Results obtained in the beginning of the investigation period are indicated within parentheses

Allergen	Positive Group I $n=20$ (%)	Positive Group II $n=15$ (%)
BLG	85	7
Unboiled milk	80 (50)	7 (20)
Boiled milk	60	7
Soy bean	50	0

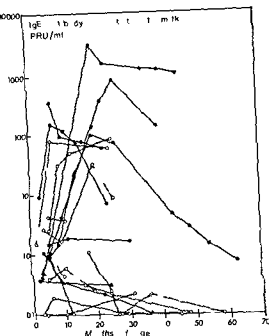


Fig 1 IgE antibody concentrations to milk Milk RAST positive infants with unchanged or possibly d creased sensitivity (●) and with definitely decreased sensitivity or complete tolerance (O) at the end of the follow up

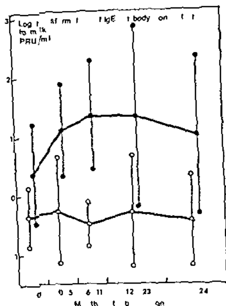


Fig 2 IgE antibody concentrations to milk in relation to development of milk tolerance Milk RAST positive infants with unchanged or possibly decreased sensitivity (●) and with definitely decreased sensitivity or complete tolerance (O) Geometric mean geometric mean ±S D

RESULTS

In *Group I* *one* third of the children had onset of symptoms already by their first few meals of cow s milk *formula* The most frequent symptoms were urticaria (71%) and diarrhoea (64%) The duration of symptoms varied from 2–24 hours In Group II the onset of symp toms was generally more insidious and mainly confined to the gastrointestinal tract Most hildren in this group developed a clinical pic ure of malabsorption

Clinical course

During the follow up period several children had recurrent episodes of allergic *symptoms* even on a milk free diet

In Group I asthma occurred in 25% and ec zema in 43% but in Group II only one child had such *symptoms* Furthermore Group I children had additional food sensitivites with reactions to egg (50%) fish (23%) pea (14%) soy bean (14%) and cereals (11%) In Group II only two children developed other food sensi tivites Soy bean formulas were initially *well* tolerated by all children but during the follow up four children of Group I and two children of Group II had adverse reactions to such for mulas including eruption of *eczema* *increased* itching and diarrhoea

Five *children seemed to be* sensitive enough to react to the trace amounts of cow s milk *protein present* in breast milk when the moth er ingested milk In two cases skin prick tests with the mothers breast milk were positive but turned negative when repeated with breast milk from the same mother off cow s milk Most children in both groups tolerated increased amounts of milk at the end of the follow up and complete tolerance was found in 29% in Group I and 74% in Group II (Table 1)

The serum IgE concentrations At onset of symptoms significantly higher serum IgE lev els were found in Group I (geometric mean 103 7 kU/l) than in Group II (geometric mean

Table 3 *The relationship between the results of skin prick tests and RAST tests with milk and soy bean in 20 children of Group I attending the final follow up investigation*

Allergen	Prick test result		RAST result		
				Positive	
			Negative	0.2–5 PRU	>5 PRU
Milk	Negative	4	3	1	0
	+	2	1	1	0
	++	7	2	2	3
	+++	7	0	2	5
Soy bean	Negative	10	8	2	0
	+	2	1	1	0
	++	3	1	1	1
	+++	5	1	1	3

16.2 kU/l) ($p<0.01$) and the levels increased further during the follow up. Even slightly elevated IgE levels at onset seemed to be predictive of rising titers of specific IgE antibodies to milk.

Skin prick tests

At the onset of the follow up period 68% of the children of Group I and 21% of Group II had positive skin prick tests with cow's milk.

At the final follow up examination, attended by 20 children of Group I and 15 children of Group II, the former showed an increased frequency of positive skin tests with milk and the reactions seemed to be stronger in most cases. The skin reactivity was greatest for BLG and greater for unboiled compared to boiled milk (Table 2). At the same time 50% of the children in Group I had positive skin prick tests with soy bean including all four children who had symptoms such as eruption of eczema or increased itching after intake of a soy bean formula. In Group II only one child had a positive skin prick test with milk and none with soy bean. Three children, who originally were prick test positive with milk, were all negative and had developed complete tolerance to milk.

Delayed skin reactions were reported by the parents of Group II in 6 patients compared to one in Group I. Two of the children had erythema, swollenness and pruritus at the site of the puncture 2–4 hours after prick tests with BLG. Four children had similar reactions 2-1? hours after prick skin tests with soy bean, two of whom had diarrhoea provoked by a soy bean formula. None of the children with delayed reactions had immediate prick skin test reactions or a positive RAST.

RAST tests

At the onset of the investigation period 71.4% of the children in Group I had positive RAST tests to milk, compared to 10.5% in Group II. The titers were significantly higher in Group I (arithmetic mean 19.6 PRU/ml, range 0.2–3300 PRU/ml) than in Group II (arithmetic mean 0.37 PRU/ml). There was no correlation between the duration of cow's milk feeding and the RAST titers. In children with a positive RAST to milk the titers increased significantly during the follow up ($p<0.05$) and even two children with initially negative RAST developed positive RAST to milk. The RAST titers often showed a peak, and thereafter declined (Fig. 1).

During the investigation period the IgE antibody titers to milk were significantly lower in milk RAST positive children who developed complete tolerance or definitely decreased sensitivity ($p<0.05$) compared to milk RAST positive children with unchanged or possibly decreased sensitivity (Fig. 2).

Positive RAST's to soy bean were found in 61% in Group I (arithmetic mean 19.0 PRU/ml, range 0.22–165 PRU/ml) and in only one patient in Group II (0.64 PRU/ml), one third of them already had positive RAST's at onset of the investigation period. All children had also positive RAST's to milk and the titers to soy bean developed in a similar manner to the titers to milk. The correlation of RAST to the skin prick test was good, as shown in Table 3. One of the two children included in the

sensitivity had a normal serum IgE level nega tive skin prick tests and negative RAST The other had an elevated IgE level (180 kU/l) and positive RAST to milk and soy bean

DISCUSSION

The significance of reagins in cow s milk sensitivity has been disputed and the frequency of IgE antibodies to milk in this condition has varied considerably in different reports (3 7 11) In this study IgE antibodies to milk were detected in high frequency in children with immediate type reactions in the skin and gastrointestinal tract after cow s milk intake Several children developed additional sensitivity to other foods animal danders and pollens and most children had a family history of allergic disease which indicate an atopic predisposition in this group

It is now evident that atopic children may have IgE antibodies to milk without any obvious symptoms (6 5) Therefore symptoms cannot be attributed to IgE antibodies alone A variety of additional factors such as gut permeability antibodies with blocking capacity the stability of mastcells and the susceptibility of the shock organs to released mediators determine if symptoms will occur or not

One third of the children in Group I had no detectable IgE antibodies to milk This could indicate non reaginic mechanisms or the occurrence of IgE antibodies to allergenic determinants not present in the cow s milk preparation used e g degradation products (18) Absence of IgE antibodies to milk in serum because of complete fixation of such antibodies to mast cells seems unlikely

In this study as in others RAST and skin prick test reactions revealed IgE antibodies to milk with the same accuracy (2) One advantage of RAST is that potentially dangerous challenge tests can be avoided in patients who have shown anaphylactic reactions

Most children developed an increased tolerance and none reported an increased sensitivity to cow s milk

With a few exceptions children with low antibody levels developed tolerance more rapidly than those with high levels Such a form of low degree transient cow s milk allergy is probably common and often overlooked (19) However the initial milk IgE antibody titers short after onset did not seem to be of prognostic value Several children with low initial titers later developed high titers

The high frequency of IgE antibodies to soy bean indicates that soy bean is a potent immunogen The low clinical significance might indicate an efficient gastrointestinal barrier to soy bean allergen

In contrast to another study (7) children with delayed reactions to cow s milk (Group II) seldom had IgE antibodies or positive skin tests to milk The reaction type to challenge in these children was more suggestive of a type III reaction if immunological at all

In conclusion cow s milk sensitivity remains a clinical diagnosis based on the outcome of elimination and re introduction of cow s milk in the diet However it is now possible to discern a distinct entity of cow s milk allergy based on reaginic mechanisms in individuals predisposed to allergic disease

ACKNOWLEDGEMENTS

This work was supported by the Swedish Medical Research Council (grants no 16X 105 16P-4910) The skilful technical assistance of Ingegerd van Katwijk and Britt Ytterberg is gratefully acknowledged

REFERENCES

1 Bachmann K D & Dees S C Milk allergy II Observations on incidence and symptoms of allergy to milk in allergic infants *Pediatrics 20 400 1957*
2 Bock S A Buckley J Holst A & May C D Proper use of skin test with food extracts in the diagnosis of hypersensitivity to food in children *Clin Allergy 7 375 1977*
3 Brenchley P E C IgE in milk allergy *Postgrad Med J 51 76 Suppl 3 1975*
4 Ceska M & Lundkvist U A new and simple radioimmunoassay method for determination of IgE *Immunochemistry 9 1021 197*

5 Dannaeus A Johansson S G O Foucard T & Öhman S Clinical and immunological aspects of allergy in childhood I Estimation of IgG IgA and IgE antibodies to food antigens in children with food allergy and atopic dermatitis *Acta Paediatr Scand* *66* 31 1977
6 Foucard T A follow up study of children with asthmatoid bronchitis I Skin test reactions and IgE antibodies to common allergens *Acta Paediatr Scand* *62* 633 1973
7 Fällström S P Ahlstedt S & Hansson L Å Specific antibodies in infants with gastrointestinal intolerance to cow s milk protein *Int Arch Allergy Appl Immunol* *56* 97 1978
8 Gerrard J W MacKenzie J W A Goluboff N Garson J Z & Maningas C S Cow s milk allergy Prevalence and manifestations in an unselected series of newborns *Acta Paediatr Scand* Suppl 234 1973
9 Goldman A S Cow milk sensitivity A review in Food and Immunology *Swedish Nutrition Symp* *VIII* 1977 p 99
10 Johansson S G O Bennich H & Berg T In vitro diagnosis of atopic allergy III Quantitative estimation of circulating IgE antibodies by the radioallergosorbent test *Int Arch Allergy Appl Immunol* *41* 443 1971
11 Kletter B Gery I Freier S Noah Z & Davies M A Immunoglobulin E antibodies to milk proteins *Clin Allergy* *1* 249 1971
12 Matthews T S & Soothill J F Complement activations after milk feeding in children with cow s milk allergy *Lancet* *II* 893 1970
13 Parish W E Detection of reaginic and short term sensitizing anaphylactoid antibodies to milk in sera of allergic and normal persons *Clin Allergy* *1* 369 1971
14 Peterson R D A & Good R A Antibodies to cow s milk—their presence and significance *Pediatrics* *31* 209 1963
15 Rothberg R M & Farr R S Incidence and amount of antibovine serum albumin (BSA) and anti alpha lactalbumin (ALA) in serum of children and adults *Clin Res* *10* 219 1962
16 Savilahti E Immunochemical study of malabsorption syndrome with cow s milk intolerance *Gut* *14* 491 1973
17 Scheinmann P Gendrek D Charles J & Paupe J Value of lymphoblast transformation test in cow s milk protein intolerance *Clin Allergy* *6* 515 1976
18 Spies J R Stevan M A & Stein W J The chemistry of allergens XXI Eight new antigens generated by successive pepsin hydrolyses of bovine β lactoglobulin *J Allergy Clin Immunol* *50* 82 1972
19 Walker Smith J Cow s milk protein intolerance Transient food intolerance of infancy *Arch Dis Child* *50* 347 1975

Submitted Aug 10 1978
Accepted Nov 7 1978

(A D) The Blood Centre
University Hospital
S 750 14 Uppsala
Sweden

Acta Pædiatr Scand 68 383–387 1979

COW'S MILK ALLERGY INCIDENCE AND PATHOGENETIC ROLE OF EARLY EXPOSURE TO COW S MILK FORMULA

G STINTZING and R ZETTERSTRÖM

From the Department of Paediatrics Karolinska Institutet St Goran s Children s Hospital Stockholm Sweden

ABSTRACT Stintzing G and Zetterstrom R (Department of Paediatrics St Goran s Children s Hospital Stockholm Sweden) Cow s milk allergy incidence and pathogenetic role of early exposure to cow s milk formula Acta Paediatr Scand 68 383 1979 —A study was performed in infants under the age of 12 months born during 1974 and admitted to St Goran s Children s Hospital with symptoms suggestive of cow s milk allergy (CMA) The aims of the study were to determine the role of early exposure to cow s milk formulas as a predisposing factor to CMA and to estimate the incidence of CMA in infancy Twenty five infants fulfilled the criteria for CMA Available records were reviewed and a careful history was obtained from the mothers on two occasions The patient group was compared with a control group Sixteen of the 25 infants were exposed to cow s milk protein during their first week in the nursery for newborns 6 were exposed before the end of the fourth week of life and 3 infants were apparently not exposed All infants were breast fed 3 to 26 weeks before re exposure and occurrence of symptoms Infants with CMA were given cow s milk formulas during their first 4 weeks of life significantly more often than infants in the control group ($p<0.01$) The incidence of CMA was approximately 1 200 The first 4 weeks after birth seem to be a particularly vulnerable period Hence in order to prevent CMA infant formula should not be given—even occasionally—during this period

KEY WORDS Cow s milk allergy neonatal feeding

Cow s milk allergy (CMA) seems to be more prevalent in societies where breast feeding is abandoned and replaced by artificial feeding at an early age As in all allergic disorders an episode of sensitization must precede the onset of allergic manifestations Wernstedt reported in 1910 that infants who developed anaphylactic reactions to cow s milk were fed cow s milk shortly after birth Recently it has been suggested that the pathogenetic mechanism is related to a transient defect in the immune apparatus in early life which is primarily related to secretory IgA of the gut (10) This leads to defective assimilation of the antigen e g of cow s milk protein which initiates the allergic process Although it has been discussed that sensitization may even take place *in utero* (6) the first weeks after birth seem to be a particularly vulnerable period from this point of view

The reported incidence of CMA varies widely (0 3–7 5%) (2 5) There are many possible explanations for this discrepancy e g lack of common clinical criteria and different feeding habits in early life

Two brands of infant formulas are currently available in Sweden both are based on cow s milk and have a similar composition They are both processed by spray-drying and have an average protein content of 13 4 g/l and a casein–whey protein ratio of 40 60 (energy 2 900 kJ/l fat 35 g/l of which linoleic acid 6 g/l lactose 72 g/l)

This study was undertaken to elucidate the possible pathogenetic role of early exposure to infant formulas in the pathogenesis of CMA and to estimate the incidence of CMA in infancy

PATIENTS AND METHODS

Children under the age of 1 months admitted to St Göran s Children s Hospital with symptoms suggestive of CMA and who were born during 1974 were investigated The following data were obtained on admission case history physical examination routine analyses of blood

5 Dannaeus A Johansson S G O Foucard T & Öhman S Clinical and immunological aspects of allergy in childhood I Estimation of IgG IgA and IgE antibodies to food antigens in children with food allergy and atopic dermatitis Acta Paediatr Scand 66 31 1977
6 Foucard T A follow up study of children with asthmatoid bronchitis I Skin test reactions and IgE antibodies to common allergens Acta Paediatr Scand 62 633 1973
7 Fällström S P Ahlstedt S & Hansson L Å Specific antibodies in infants with gastrointestinal intolerance to cow s milk protein Int Arch Allergy Appl Immunol 56 97 1978
8 Gerrard J W MacKenzie J W A Goluboff N Garson J Z & Maningas C S Cow s milk allergy Prevalence and manifestations in an unselected series of newborns Acta Paediatr Scand Suppl 234 1973
9 Goldman A S Cow milk sensitivity A review in Food and Immunology Swedish Nutrition Symp XIII 1977 p 99
10 Johansson S G O Bennich H & Berg T In vitro diagnosis of atopic allergy III Quantitative estimation of circulating IgE antibodies by the radioallergosorbent test Int Arch Allergy Appl Immunol 41 443 1971
11 Kletter B Gery I Freier S Noah Z & Davies M A Immunoglobulin E antibodies to milk proteins Clin Allergy 1 249 1971
12 Matthews T S & Soothill J F Complement activations after milk feeding in children with cow s milk allergy Lancet II 893 1970
13 Parish W E Detection of reaginic and short t sensitizing anaphylactoid antibodies to milk in ser allergic and normal persons Clin Allergy 1 , 1971
14 Peterson R D A & Good R A Antibodie cow s milk—their presence and significance Pe rics 31 209 1963
15 Rothberg R M & Farr R S Incidence and am of antibovine serum albumin (BSA) and anti al lactalbumin (ALA) in serum of children and adı Clin Res 10 219 1962
16 Savilahti E Immunochemical study of malabs tion syndrome with cow s milk intolerance Gat 491 1973
17 Scheinmann P Gendrek D Charles J & Pa J Value of lymphoblast transformation test in co milk protein intolerance Clin Allergy 6 515 197
18 Spies J R Stevan M A & Stein W J Th ch istry of allergens XXI Eight new antigens gener by successive pepsin hydrolyses of bovin β la globulin J Allergy Clin Immunol 50 82 197
19 Walker Smith J Cow s milk protein intolera Transient food intolerance of infancy Arch Dis Ch 50 347 1975

Submitted Aug 10 1978
Accepted Nov 7 1978

(A D) The Blood Centre
University Hospital
S 750 14 Uppsala
Sweden

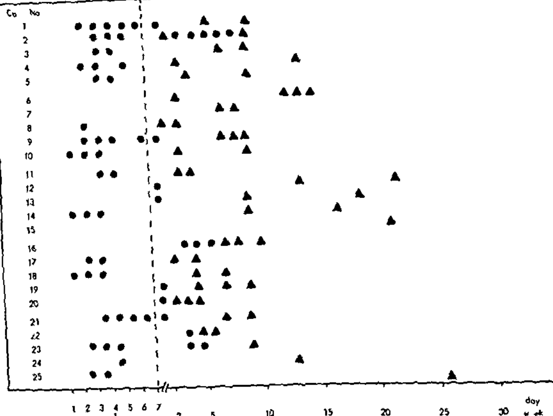


Fig. 1. Patients with CMA. ● The day or week the patient was given any infant formula without reacting to it. ▲ the week an allergic symptom was noted.

Sixteen patients were given the formula on one or more occasions on the second to the seventh day, most frequently on the fourth day. The amount of formula given on any day ranged from 10 to 300 ml. In 2 cases the patient received the formula only once. In 10 instances the mother did not know or had forgotten about this early exposure to cow's milk protein. All 25 babies were fully or at least partly breast fed at the time of discharge from the newborn nursery.

Of the remaining 9 babies, 6 were given infant formula within the first 4 weeks of life. In only 3 cases was no evidence obtained of any previous exposure to cow's milk protein. Fig. 1 also shows that one patient developed symptoms on the first re-exposure to cow's milk protein as late as the 26th week.

The symptoms are listed in Table 2. Twenty one of the patients (84%) had gastrointestinal and 10 (40%) cutaneous symptoms. In 4 cases the latter were the only manifestations of CMA. Four patients (16%) developed respiratory symptoms. Shock was seen in 2 patients. In 23 of 25 patients, no untoward reactions were observed following ingestion of the soy based formula. The 2 infants who developed symptoms on soy formula tolerated Nutramigen[8] well.

The control group consisted of 52 infants; 48 were breast fed at the time of discharge from the newborn nursery and the remaining 4 infants were not. In 47 cases it was possible to contact the mothers one year after for a follow up dietary history. The amount of formula given on any day varied from 10 to

Table 1 *Patients with CMA*

No of patients	Boys/ girls	Age at first exposure (mean and range)	No of infants exposed in the newborn nursery (day 1–7)	Age at onset of symptoms (mean and range)	Age on admission (mean and range)
25	16/9	1½ w 2 d – 4 w	16	7½ w 3–26 w	10½ w 5–26 w

including immunoglobulins skin test or intradermal test to cow s milk and a careful dietary history from each mother Records from the newborn nursery well baby clinics and other hospitals were reviewed for information concerning previous health status weight gain and dietary history Regular follow up in the out patient department consisted of a physical examination and a dietary history Note was made of the day or week the patient was given infant formula and the week when symptoms occurred

The criteria for CMA in this study are identical with those used by Goldman (7) 1) subsidence of symptoms after dietary elimination of cow s milk 2) recurrence of symptoms within 48 hours after challenge with cow s milk and 3) positive reactions showing similar onset duration and clinical features to three such challenges Thus each patient was challenged one or two times with full cow s milk and on one occasion with the same formula that induced symptoms prior to admission The patient was always under professional supervision when challenged The challenge started with 2 5 ml followed at 2 hour intervals with 5 10 20 40 ml etc until the ordinary amount for the patient s feed was reached or stopped if some reaction was registered

On admission and after challenge the patient was given a soy based formula ProSobee® Sobee® or secondarily a casein hydrolysate Nutramigen® unless he could be fully breast fed Symptoms were listed under three main headings 1) gastrointestinal e g vomiting and diarrhoea 2) cutaneous e g rash urticaria and eczema and 3) respiratory e g rhinitis asthmatic symptoms Shock was noted separately

The control group consisted of the last baby born on a Monday and the first baby born on a Thursday in one of the nurseries for the newborn during the first half year of 1974 Fifty two babies 28 boys and 24 girls were included Records from the newborn nursery well baby clinics and other hospitals were reviewed The mother was interviewed regarding the dietary and health history of her baby when he/she had reached an age of about 12 months In a few cases the mother and her baby were seen in the out patient department

RESULTS

Twenty five infants fulfilled the criteria for CMA They were all born at term with appropriate weight for gestational age Prior to the onset of allergic symptoms all infants were healthy Routine blood analyses including serum immunoglobulins were normal

Sixteen of the infants were boys and 9 were girls In 16 cases the patient received a cow s milk formula in the newborn nursery The mean age at onset of symptoms was 7½ weeks and on admission 10½ weeks (see Table 1)

Fig 1 shows the day or the week the baby received formula with or without reacting to it

Table 2 *Symptoms of patients with CMA*

Case no	Symptoms: Gastro-intestinal	Cutaneous	Respiratory
1	+		
2		+	+
3	+		
4	+	+	
5	+	+	
6		+	
7	+		+
8	+		
9	+		
10	+		
11	+		+
12	+	+	
13	+		
14	+		
15	+		
16	+	+	
17	+		
18	+	+	+
19	+	+	
20	+		
21	+		
22		+	
23	+		
24	+		
25		+	
	21	10	4
	84%	40%	16%

Symptoms of shock

infants who may have had this symptom as the only manifestation of CMA It is also possible that a number of infants have mild symptoms of CMA which subside spontaneously Since no such infants have ever come to this hospital none are included in this study

The fact remains that the occasional and often forgotten exposure to cow s milk protein seems to play an important role in the pathogenesis of CMA as recently suggested by Buisseret (1) Altered composition of infant formulas and changed feeding habits of newborns and small infants may play an important role as well The most important prophylactic measure must be encouragement of breast feeding from the first day of life and strict avoidance of unnecessary and occasional bottle feeding with cow s milk based formulas at least during the first month of life

REFERENCES

1 Buisseret P D Common manifestations of cow s milk allergy in children *Lancet* i 304 1978
2 Collins Williams C The in idence of milk allergy in pediatric practice *J Pedi itr 48* 39 1956
3 Dannaeus A Johansson S G O & Foucard T Clinical and immunological aspects of food allergy in childhood II *Acta Paediatr Scand 67* 497 1978
4 Gerrard J W Lubos M C Hardy L W Holmlund B A & Webster D Milk allergy clinical picture and familial incidence *Can Med Assoc J 97* 780 1967
5 Gerrard J W Mackenzie J W A Goluboff N Garson J Z & Maningas C S Cow s milk allergy Prevalence and manifestations in an unselected series of newborns *Acta Paediatr Scand* Suppl *234* 1973
6 Glaser J & Johnstone D C Prophylaxis of allergic disease in the newborn *JAMA 153* 620 1953
7 Goldman A S Anderson D W Sellers W A Saperstein S Kniker W T & Halpern S R Milk allergy I Oral challenge with milk and isolated milk proteins in allergic children *Pediatrics 32* 425 1963
8 Goldman A S Sensitivity to cow s milk *In* L Hambraeus L Å Hanson & H McFarlane (eds) *Food and Immunology* Almqvist & Wiksell Stockholm 1977 p 99
9 Savilahti E Immunochemical study of the malabsorption syndrome with cow s milk protein intolerance *Gut 14* 491 1973
10 Soothill J F Immunodeficiency allergy and infant feeding *In* L Hambraeus L Å Hanson & H McFarlane (eds) *Food and Immunology* Almqvist & Wiksell Stockholm 1977 p 88
11 Wernstedt W Zur Frage der Kuhmilchidiosynkrasie im Säuglingsalter *Monatsschr Kinderheilkd 9* 345 1910

Submitted July 14 1978
Accepted Sept 22 1978

(G S) Department of Paediatrics
S t Gorans Sjukhus
Box 12500
S 11281 Stockholm
Sweden

300 ml. In 8 of 19 cases the mother was unaware of, or had forgotten that her baby had been given formula in the newborn period. Another 6 patients were either occasionally or regularly given some infant formula during the first 4 weeks of life. Except for one case that could not be followed up, no baby in the control group had shown symptoms attributable to CMA during the first year of life.

In comparison with infants in the control group the patients with CMA were given infant formula significantly more often during their week long stay in the newborn nursery as well as during their first 4 weeks of life (χ^2 analysis $p<0.05$ for first week and $p<0.01$ for first 4 weeks of life).

Five of the patients were cared for in the same newborn nursery during the first half of 1974 as the babies in the control group. During this period a total of 1 024 babies were admitted to this nursery, which gives an incidence of 0.49% for CMA for this nursery. Some 4 311 babies were born in the entire area from which all patients are admitted to this children's hospital. Since the total number of patients with CMA was 25, the calculated incidence for the area is 0.58%. This means that approximately one baby in 200 will develop CMA during the first year of life.

DISCUSSION

In this study we found that of 25 infants who developed CMA, 22 received cow's milk formula on at least one occasion during the first 4 weeks of life and 16 of them received some formula even during the first week after birth. It is also of interest that all babies were at least partly breast fed for some time before the onset of symptoms of CMA. Our observations thus accord with those of Wernstedt (11) which were reported in 1910. Collins-Williams (2) likewise concludes that it is probably more deleterious to give infant formulas just occasionally rather than regularly in early life. In Sweden, newborns usually remain in the newborn nursery during the first week of life. It is therefore noteworthy that many mothers did not know or had forgotten that their baby had been given infant formula during this period. In the control group we also found that it was a fairly common practice to give infant formulas occasionally in the newborn nursery. This apparently occurs because the staff is uncertain whether the mothers will have enough breast milk and because they want to induce rapid weight gain. This ambition may represent an extension of the practice of "early feeding" of small for gestational age babies to full term healthy newborns. However, it is not possible to exclude exposure to cow's milk protein even if the baby was never given formula deliberately. Since foods ingested by the mother can cause allergic reactions in breast fed babies (4) this might explain how the 3 cases who had no known previous direct exposure to cow's milk protein developed CMA. Sensitization *in utero* might be another possibility (6).

All of the principal proteins in cow's milk have been incriminated in the pathogenesis of CMA (7) and complex immunologic mechanisms (9), including IgE antibodies (3), are involved in the allergic reaction. The reduced content of casein in relation to whey proteins as is the case with so called humanized infant formulas currently in use and the processing itself may render formulas more allergenic than cow's milk.

It is generally believed that the incidence of CMA has increased in recent years. Our incidence of about 0.5% is low in comparison to that reported by Gerrard et al. (5), i.e. 7.5%, but using Goldman and co-workers' criteria it is similar to that suggested by the same author in 1977 for the United States, viz. 0.5–1.0%. However, figures for incidence are somewhat difficult to compare because of various factors related to selection. Another reason for the wide range of the reported incidence is that different criteria are used for the diagnosis of CMA in different studies. Due to difficulties in evaluating such ill defined symptoms as colic we have been forced to exclude

infants who may have had this symptom as the only manifestation of CMA It is also possible that a number of infants have mild symptoms of CMA which subside spontaneously Since no such infants have ever come to this hospital none are included in this study

The fact remains that the occasional and often forgotten exposure to cow s milk protein seems to play an important role in the pathogenesis of CMA as recently suggested by Buisseret (1) Altered composition of infant formulas and changed feeding habits of newborns and small infants may play an important role as well The most important prophylactic measure must be encouragement of breast feeding from the first day of life and strict avoidance of unnecessary and occasional bottle feeding with cow s milk based formulas at least during the first month of life

REFERENCES

1 Buisseret P D Common manifestations of cow s milk allergy in children *Lancet I* 304 1978
2 Collins Williams C The incidence of milk allergy in pediatric practice *J Pediat 48* 39 1956
3 Dannaeus A Johansson S G O & Foucard T Clinical and immunological aspects of food allergy in childhood II *Acta Paediat Scand 67* 497 1978
4 Gerrard J W Lubos M C Hardy L W Holmlund B A & Webster D Milk allergy clinical picture and familial incidence *Can Med Assoc J 97* 780 1967
5 Gerrard J W Mackenzie J W A Goluboff N Garson J Z & Maningas C S Cow s milk allergy Prevalence and manifestations in an unselected series of newborns *Acta Paediatr Scand* Suppl *234* 1973
6 Glaser J & Johnstone D C Prophylaxis of allergic disease in the newborn *JAMA 153* 620 1953
7 Goldman A S Anderson D W Sellers W A Saperstein S Kniker W T & Halpern S R Milk allergy I Oral challenge with milk and isolated milk proteins in allergic children *Pediatrics 32* 425 1963
8 Goldman A S Sensitivity to cow s milk *In* L Hambraeus L Å Hanson & H McFarlane (eds) *Food and Immunology* Almqvist & Wiksell Stockholm 1977 p 99
9 Savilahti E Immunochemical study of the malabsorption syndrome with cow s milk protein intolerance *Gut 14* 491 1973
10 Soothill J F Immunodeficiency allergy and infant feeding *In* L Hambraeus L Å Hanson & H McFarlane (eds) *Food and Immunology* Almqvist & Wiksell Stockholm 1977 p 88
11 Wernstedt W Zur Frage der Kuhmilchidiosynkrasie im Sauglingsalter *Monatsschr Kind rheilkd* 9 345 1910

Submitted July 14 1978
Accepted Sept 2 1978

(G S) Department of Paediatrics
S t Gorans Sjukhu
Box 12500
S 11281 Stockholm
Sweden

Acta Pædiatr Scand 68 389-396 1979

HUMAN COLOSTRAL AND BREAST MILK CELLS

A Light and Electron Microscopic Study

FAITH C S HO ROSAMOND L C WONG
and JOHN W M LAWTON

From the Departments of Pathology and Obstetrics and Gynaecology University of Hong Kong Hong Kong

ABSTRACT Ho F C S Wong R L C and Lawton J W M (Departments of Pathology and Obstetrics and Gynecology University of Hong Kong) Human colostral and breast milk cells a light and electron microscopic study Acta Paediatr Scand 68 389 1979 —Colostrum and breast milk samples were obtained from 74 women 18 of whom gave sequential samples The mean total leukocyte count in colostrum was 3190 cells/mm³ Proportions of macrophages polymorphs and lymphocytes varied widely macrophages usually predominated Serial sampling showed (1) a small fall in total counts through delivery (2) a fall in total counts and the proportion of PMNs at the onset of lactation (3) after 1 to 2 weeks of lactation the appearance of cytoplasmic fragments together with epithelial cells which later constituted the main cell type It was estimated that the total number of leukocytes available to the neonate remained approximately constant during the first 2 weeks of lactation and fell thereafter Functionally morphologically and histochemically macrophages in colostrum and breast milk resembled macrophages elsewhere Their ultrastructure was characterised by filiform surface projections numerous endocytic vacuoles and lipid droplets in the cytoplasm

KEY WORDS Colostrum breast milk leukocytes macrophages lactation electron microscopy

The presence of leukocytes in human colostrum and breast milk has long been recognized but has received scant attention These cells comprise macrophages polymorphonuclear leukocytes (PMNs) and lymphocytes (11 12 20) Results of recent studies have indicated that they may play an important role in transferring specific and non specific host resistance factors to the neonate The mononuclear cells in colostrum may contribute specifically by synthesizing IgA antibodies (14) particularly against enteric bacteria (1) and non specifically by synthesizing complement (14) lysozyme (11) and interferon (5 10) Lymphocytes in colostrum and breast milk respond to mitogens recall antigens and allogeneic cells (4 17 20) and there is evidence that a component of breast secretions can passively transfer cell mediated immunity to the infant (13) although this may not be long lasting (15) The macrophages and PMNs may protect the neonate by their capacity to ingest and to kill microorganisms (18) they can ingest *C albicans* and *E coli* as avidly as blood leukocytes but their killing capacity is much less (7) There is also evidence that breast milk macrophages may act as a vehicle of immunoglobulin transport (19)

In early postpartum colostrum the total leukocyte counts are comparable to those in peripheral blood counts ranging from 500 to 8 000/mm³ have been found macrophages making up 30 to 60% of the cells As lactation is established the cell concentration drops while the proportion of macrophages rises (11)

In this study of a predominantly Chinese population we set out to extend this knowledge by doing serial studies of cell counts in colostrum and breast milk through parturition and established lactation and to further characterize the cells by cytochemical stains and

by electronmicroscopy This formed part of a wider study of the function of these cells and their possible significance to the neonate

MATERIALS AND METHODS

Colostrum and breast milk samples from 74 women (67 Chinese 6 Caucasian 1 Indian) were obtained by manual expression into sterile plastic Universal bottles Fifty six donors gave one sample only Sequential samples were donated by 18 women 3 of them up to 6 months of lactation The data referred to below as data from random samples was collated from all individual samples and appropriately timed sequential samples In addition all data derived from sequential samples was tabulated separately (serial samples)

Preparation of cells

Colostrum diluted 1/2 in Hanks Balanced Salt Solution (HBSS) and undiluted breast milk was spun at 100×g for 10 min The cells were washed 2× in HBSS prior to suspension in medium RPMI 1640 containing HEPES/bicarbonate buffer penicillin and streptomycin and 20% heat inactivated human AB serum Fifty thousand cells in 1 to 2 ml were spun onto a circular glass coverslip (18 mm diam) at 100×g for 5 min The coverslip was air dried prior to Wright s staining for differential counts

Cytochemistry

Cells prepared by cytocentrifugation were stained for non specific esterase (using α naphthyl acetate as substrate) chloroacetate esterase (22) acid phosphatase and myeloperoxidase (MPO) and with Oil Red O

Phagocytosis

Cells suspended in LGM were exposed to a suspension of polystyrene particles (0 05% 0 8 μ diam) at room temperature for 30 min To assess phagocytosis of bacteria cells were suspended in HBSS containing 20% fresh AB serum as a source of opsonins heat killed *S aureus* or *E coli* were added (approximately 10 organisms per cell) and the mixture was incubated at 37 C for 30 min in a shaking water bath

Electron microscopy

Aliquots of approximately 1×10^6 washed cells were fixed in suspension in 2 5% glutaraldehyde for 4–6 hours post fixed in 1% phosphate buffered osmium tetroxide and to the centrifuged pellet were added a few drops of 2% agarose gel at 60 C The cells in agarose were pipetted onto a dental wax plate and when set the gel was cut into small cubes These were dehydrated in increasing concentrations of ethanol and transferred through propylene oxide to Epon 812 Thin sections of selected blocks were stained with uranyl acetate and lead nitrate and examined in a Philips EM300 electron microscope In 3 of the 8 samples studied separate aliquots of cells were first exposed to *S aureus* then processed as described above

RESULTS

Cell morphology

Mononuclear phagocytes (MPs) were arbitrarily classified as small macrophages 8 20 μ in diameter and large macrophages 20 40 μ in diameter (including the corpuscles of Donne) but the cells display a continuum of appearances from the smallest (with scanty cytoplasm resembling lymphocytes) to the largest with copious vacuolated cytoplasm (Fig 1) Occasional cells closely resembled blood monocytes Large macrophages often had ingested cellular material in the cytoplasm and were sometimes multinucleate

Both small and large macrophages were shown to be actively phagocytic for *S aureus* *E coli* and polystyrene particles

Epithelial cells were typically 15 to 20 μm in diameter had deeply basophilic cytoplasm and a tendency to occur in clumps of 2 to 4 cells (Fig 2a) They were not phagocytic *Cytoplasmic fragments* showed staining characteristics similar to the epithelial cell cytoplasm (Fig 2b)

Polymorphs were mostly neutrophilic an occasional eosinophil was seen Often large cytoplasmic vacuoles were present pushing the nucleus to the periphery PMNs were less avidly phagocytic for bacteria and polystyrene particles than were the macrophages

Lymphocytes were morphologically unremarkable

Cell counts on random samples

Random sampling showed little change in absolute counts or in proportions of leukocyte types from the prepartum to the immediate post partum period (Table 1) As lactation became established the total count dropped steeply to a mean of about 400/mm³ at the end of the first week while the mean proportion of macrophages rose from 49% to 72% Equally striking was the reciprocal fall in numbers of polymorphs as the milk flow increased The mean percentage of lymphocytes was fairly







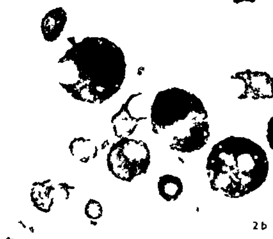


Fig. 1 (*a*) Small macrophages (*arrowed*) and PMNs (*P*) in prepartum colostrum. The small macrophages have moderately basophilic finely vacuolated cytoplasm and occasional azurophilic granules. The nucleus is round, oval, reniform or irregular and eccentrically placed with moderately condensed chromatin containing 1 to 3 small nucleoli.

(*b*) A large macrophage in prepartum colostrum. The cytoplasmic vacuoles are numerous and prominent giving the cell its characteristic foamy appearance. Foamy macrophages not uncommonly have a centrally placed nucleus, see Fig. 2A. (Wright's stain ×1250.)

Fig. 2 (*a*) A clump of 4 epithelial cells and a foamy macrophage in 3 week breast milk. The epithelial cells have deeply basophilic cytoplasm, a round eccentrically placed nucleus with smooth or finely fenestrated chromatin. Clear secretory vacuoles in the cytoplasm vary greatly in number and size.

(*b*) Epithelial cells in 3 week breast milk together with cytoplasmic fragments. Many of these fragments contained a single large vacuole. (Wright's stain ×1250.)

Table 1 *Cell counts on random samples of colostrum and early breast milk*

	Total count mean cells per mm³	Macrophages Mean %	Macrophages Mean cells per mm³	Polymorphonuclear leukocytes Mean %	Polymorphonuclear leukocytes Mean cells per mm³	Lymphocytes Mean %	Lymphocytes Mean cells per mm³
Prepartum							
3–1 weeks (n=11)	3 370 (950–11 250)	40 5 (11–68)	1 200 (760–1 920)	52 7 (13–87)	2 240 (120–9 790)	5 7 (1–19)	170 (40–[illegible]0)
7–0 days (n=28)	3 530 (750–9 400)	52 6 (9–89)	1 700 (90–6 940)	38 6 (2–83)	1 500 (80–5 360)	8 5 (0–43)	189 (0–560)
Postpartum							
0–2 days (n=16)	2 810 (100–14 000)	49 1 (8–84)	1 220 (50–4 760)	44 3 (9–69)	1 430 (55–5 040)	6 6 (2–23)	180 (2–1 8 0)
3–4 days (n=14)	1 140 (65–6 000)	48 6 (8–94)	390 (40–2 460)	44 9 (3–97)	701 (20–5 640)	5 4 (0–16)	47 (1– 09)
5–8 days (n=12)	439 (53–1 970)	72 2 (46–92)	315 (14–1 620)	20 0 (5–53)	96 (3–419)	5 3 (1–12)	27 (1–194)

Ranges of values are given in parentheses

constant in the first week postpartum (5 3% to 6 6%)

By the second week of lactation the absolute numbers of all cell types had dropped markedly probably due to a dilution effect

Counts on serial samples

Serial sampling revealed trends which were not apparent by random sampling (Table 2) From pre to post partum colostrum there was a small drop in mean total cell count a fall in

Table 2 *Cell counts on serial samples of colostrum and milk*[a]

	Total count mean cells per mm³	Macrophages Mean %	Macrophages Mean cells per mm³	Polymorphonuclear leukocytes Mean %	Polymorphonuclear leukocytes Mean cells per mm³	Lymphocytes Mean %	Lymphocytes Mean cells per mm³	Epithelial cells Mean %	Epithelial cells Mean cells per mm³
Pre partum									
7–0 days (n=7)	3 430 (1 100–7 800)[b]	66 (37–93)	2 140 (350–6 940)	21 (2–41)	560 (10–900)	11 (2–36)	240 (70–390)		
Post partum									
0–4 days (n=14)	2 840 (300–14 000)	48 (24–87)	1 300 (50–4 760)	42 (11–67)	1 350 (60–7 470)	7 3 (1–17)	207 (2–1 870)	<1	–
5–8 days (n=12)	439 (50–1 970)	72 (46–92)	315 (14–1 620)	20 (5–53)	96 (3–420)	5 3 (1–12)	27 (1–190)	75 (0–5)	<1
1–2 weeks (n=7)	69 (4–130)	75 (61–97)	52 (2–130)	56 (2–23)	4 (1–7)	2 9 (0–13)	<1 (0–1)	5 6 (0–23)	4 (0–9)
2–4 weeks (n=9)	51 (15–167)	44 (2–88)	22 (1–84)	16 (1–89)	8 (1–37)	2 2 (0–4)	1 (0–4)	36 (7–80)	23 (1–60)
1–2 months (n=5)	17 (5–30)	24 (2–38)	4 (1–11)	10 (1–25)	3 (1–8)	7 2 (0–27)	1 (0–4)	56 (53–96)	8 (5–15)
2–4 months (n=6)	16 (3–60)	11 (0–47)	5 (0–31)	14 (1–67)	2 (0–8)	2 5 (0–14)	1 (0–9)	71 (28–94)	9 (3–20)
4–6 months (n=5)	10 (3–27)	2 7 (0–6)	<1 (0–1)	4 6 (0–18)	<1 –	0 9 (0–8)	<1 –	84 (58 99)	9 (2–26)

[a] Samples donated by a total of 18 individuals

[b] Range of values in parentheses

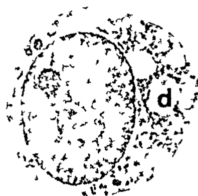


Fig 3 Epithelial cell in day 4 breast milk The cell out line is smooth and the nucleus large and ovoid There are numerous free ribosomes and some short profiles of rough endoplasmic reticulum One fat droplet (*d*) and several vesicles are seen at one pole of the cell Electron micrograph (×5 600)



Fig 4 Macrophage in prepartum colostrum with charac teristic surface projections The flattened nucleus shows moderate peripheral condensation of chromatin Two large vacuoles (V) containing cellular debris are seen Electron micrograph (×5 600)

macrophage and lymphocyte percentages and a rise in the number of PMNs

During the second week of lactation epi thelial cells were found in small numbers (0–23%) accompanied by numerous cell frag ments probably derived from the process of apocrine secretion While the absolute num bers of leukocytes continued to fall the epi thelial cells reached the highest concentration of 23 cells/mm³ late in the first month and thereafter became the predominant cell type

Cytochemistry

The macrophages stained strongly for non specific esterase (fluoride inhibited) and acid phosphatase They were usually negative for MPO and chloro acetate esterase The stain ing pattern with Oil Red O indicated that the abundant cytoplasmic vacuoles in macro pages and PMNs were lipid containing

Colostral PMNs stained weakly for both chloro acetate esterase and MPO in contrast to the strong reactions given by blood PMNs (22) The epithelial cells and cell fragments stained for non specific esterase and acid phosphatase and contained lipid droplets

Electron microscopy

Of the range of cell types seen most numerous were MPs of varying morphology and PMNs but there were occasional lymphocytes and epithelial cells (Fig 3) as well as fragments of epithelial cell cytoplasm

Cells of the MP series were identified by an indented or irregularly shaped nucleus their relatively abundant cytoplasm large veil like and smaller filiform projections from their surface membrane and the presence of endo cytic vesicles containing material of varied na ture Some vacuoles contained scanty floccu lent material whereas others had a moderately electron dense homogeneous content con sistent with lipid droplets Phagocytosed cell debris and phagosomes with dense material (residual bodies) were also seen (Fig 4) The cells containing numerous lipid droplets of uni form size (Fig 5) are probably the foam cells (Corpuscles of Donne) seen by light micro scopy Transitional forms between these cells and typical MPs and phagocytosis of *S aureus* by them (Fig 6) indicate their macrophage ori gin Other organelles were clearly seen only in the cells with fewer lipid droplets these in

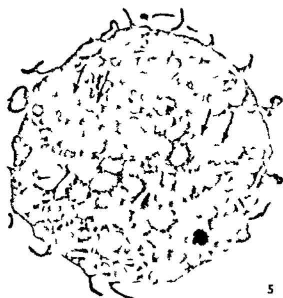






Fig 5 Macrophage in prepartum colostrum containing numerous lipid droplets some arrowed These cells are considered the equivalent of the foamy macrophages seen by light microscopy Electron micrograph (×5 600)

Fig 6 Macrophage in prepartum colostrum after incubation with heat inactivated *S aureus* The ingested cocci are arrowed This cell resembles that in Fig 5 but has larger veil like cytoplasmic projections sometimes folded back to enclose small vesicles Electron micrograph (×8 100)

Fig 7 A group of neutrophils in day 4 breast milk These cells show a heavy peripheral condensation of chromatin in their segmented nuclei Endocytic vesicles with heterogeneous contents are seen Electron micrograph (×4 600)

cluded small scattered mitochondria a usually well developed Golgi apparatus strands of rough endoplasmic reticulum near the periphery of the cell and many free ribosomes Where these cells were in contact with cytoplasmic fragments fingerlike projections and deep invaginations of the surface membrane were abundant In many fields several cells surrounded and enclosed cellular debris or large lipid globules

PMNs were characterised by their multilobed nucleus with heavy peripheral condensation of chromatin (Fig 7) Although they also contained a range of inclusions similar to the MPs none of them showed signs of transformation into foam cells Specific granules were preserved in only a few cells and degenerative changes were more advanced and more evident in PMNs than in MPs The variations observed in the preservation of fine structure of these and other cells within the same sample may be related not only to differences in their natural lifespan but also to the duration of their immersion in the rather special environment of the colostrum

Lymphocytes had a high nuclear cytoplasmic ratio a few short uniformly distributed surface projections abundant cytoplasmic

olyribosomes and a few strands of rough endoplasmic reticulum Mitochondria were usually grouped on one side of the nucleus but we did not observe further evidence of polarity in these cells as reported by Smith et al (21) These investigators allowed the cells to settle on a cover glass to facilitate interaction whereas in our work the cells were fixed in suspension However in those rare instances where lymphocytes were found in close proximity to macrophages there was some interdigitation of their cytoplasmic processes

DISCUSSION

The mean total leukocyte counts and differential counts in colostrum and early breast milk in our study are in close agreement with similar observations by other workers (11 12 20) but we found a wider range in total counts

The marked fall in the proportion of PMNs and a reciprocal rise in the proportion of macrophages with onset of lactation may explain the observation of Smith & Goldman (20) that suckling is associated with a lower proportion of PMNs in colostrum Over the first 2 weeks mean total counts fall about 50-fold while the volume of breast secretion increases from a few ml/day to about 500 ml/day Hence the total number of leukocytes ingested by the neonate remains approximately constant during this period After 2 weeks however total cell counts continue to fall while milk volume increases relatively little to about 750 ml/day (8)

The corpuscles of Donne (colostral corpuscles) are now usually classified together with the other large lipid laden foamy macrophages of colostrum and early breast milk These cells and the smaller macrophages share the characteristics of MPs found in the tissues or other body fluids they are glass adherent show ameboid movement and are actively phagocytic for fungi bacteria colloidal particles and red cells (9 11 20) in tissue culture they form epithelioid and multinucleate forms and remain avidly phagocytic for polystyrene particles and staphylococci (unpublished observations) We have shown their ultrastructure to be similar to that of macrophages found elsewhere (2 3) and they stain strongly for lysozomal enzymes On the basis of observations of human mammary gland explants in culture Papanicolaou & Maddi (16) claimed that the foamy cells of breast secretions developed from epithelial cells On the other hand their morphological functional and histochemical characteristics suggest that they derive from cells belonging to the system of mononuclear phagocytes originating in the bone marrow and entering the breast tissues by way of the blood monocyte

Colostral macrophages bear a considerable morphologic resemblance to lung alveolar macrophages as described by Cline (3) and like the lipid laden alveolar macrophages in pulmonary alveolar proteinosis (6) they have a low microbicidal capacity (7) While their microbicidal capacity appears low their phagocytic capacity is comparable to that of blood leukocytes (7) and this ability to sequester pathogens may also contribute to the protection of the breast fed neonate

ACKNOWLEDGEMENTS

We take pleasure in thanking Miss Chan Kwan Lai for her excellent technical assistance Miss Josephine Chan for her endless patience in typing the manuscript and Mrs Penny Lawton for collecting and donating numerous samples

REFERENCES

1 Ahlstedt S Carlsson B Hanson L Å & Goldblum R M Antibody production by human colostrum cells I Immunoglobulin class specificity and quantity *Scand J Immunol 4* 535 1975

Carr I *The macrophage A review of ultrastructure and function* Academic Press London 1973

3 Cline M J *The white cell* Harvard University Press Cambridge Massachusetts 1975 p 459

4 Diaz Jouanen E & Williams R C T and B lymphocytes in human colostrum *Clin Immunol Immunopathol 3* 248 1974

5 Emodi G & Just M Interferon production by lymphocytes in human milk *Scand J Immunol 3* 157 1974

6 Golde D W Termio M Finley T N & Cline M J Defective lung macrophages in pulmonary alveolar proteinosis *Ann Intern Med 85* 304 1976

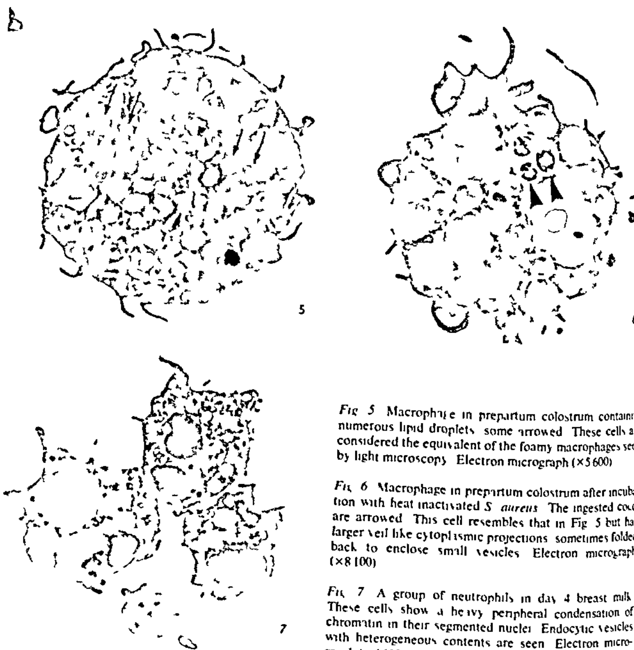


Fig 5 Macrophage in prepartum colostrum containing numerous lipid droplets some arrowed These cells are considered the equivalent of the foamy macrophages seen by light microscopy Electron micrograph (×5 600)

Fig 6 Macrophage in prepartum colostrum after incubation with heat inactivated *S aureus* The ingested cocci are arrowed This cell resembles that in Fig 5 but has larger veil like cytoplasmic projections sometimes folded back to enclose small vesicles Electron micrograph (×8 100)

Fig 7 A group of neutrophils in day 4 breast milk These cells show a heavy peripheral condensation of chromatin in their segmented nuclei Endocytic vesicles with heterogeneous contents are seen Electron micrograph (×4 600)

cluded small scattered mitochondria a usually well developed Golgi apparatus strands of rough endoplasmic reticulum near the periphery of the cell and many free ribosomes Where these cells were in contact with cytoplasmic fragments fingerlike projections and deep invaginations of the surface membrane were abundant In many fields several cells surrounded and enclosed cellular debris or large lipid globules

PMNs were characterised by their multilobed nucleus with heavy peripheral condensation of chromatin (Fig 7) Although they also contained a range of inclusions similar to the MPs none of them showed signs of transformation into foam cells Specific granules were preserved in only a few cells and degenerative changes were more advanced and more evident in PMNs than in MPs The variations observed in the preservation of fine structure of these and other cells within the same sample may be related not only to differences in their natural lifespan but also to the duration of their immersion in the rather special environment of the colostrum

Lymphocytes had a high nuclear cytoplasmic ratio a few short uniformly distributed surface projections abundant cytoplasmic

Acta Pædiatr Scand 68 397–400 1979

INCIDENCE OF COELIAC DISEASE AND TRANSIENT GLUTEN INTOLERANCE IN CHILDREN IN A SWEDISH URBAN COMMUNITY

N O BERG and T LINDBERG

From the Department of Pathology University Hospital Lund and the Department of Paediatrics Malmo General Hospital Malmo University of Lund Sweden

ABSTRACT Berg N O and Lindberg T (Department of Pathology University Hospital Lund and Department of Paediatrics Malmo General Hospital Malmo University of Lund Sweden) Incidence of coeliac disease and of transient gluten intolerance in children in a Swedish urban community Acta Paediatr Scand 68 397 1979 —The incidence of coeliac disease in children in the city of Malmo South Sweden was 1 982 during 1966 to 1975 The diagnostic criteria were flat intestinal mucosa on gluten containing diet free of symptoms and improvement in mucosal morphology on gluten-free diet and morphological and/or evident clinical relapse (three times) on gluten challenge 6 (12%) of 49 children with initially a flat mucosa still had a normal mucosa on a gluten-containing diet for two years or longer having so-called transient gluten intolerance

KEY WORDS Coeliac disease gluten intolerance intestinal biopsy

The reported incidence of coeliac disease varies considerably (4 5 6 8 13 15 19 22) This can be due to a variation in the incidence from one area to another also to the use of different criteria for the diagnosis Two reports are available from Sweden They gave an incidence of 1 6500 (1950–1962) (4) and 1 3700 (1952–1963) (8) These figures are based on retrospective studies the diagnosis being mainly determined on clinical findings

This work reports the incidence of coeliac disease in children in a Swedish urban community during a ten year period (1966–1975) The criteria for the diagnosis were (9) a flat or nearly flat mucosa in biopsy from duodenojejunal flexure on a gluten containing diet disappearance of symptoms and a definite restitution of mucosal architecture on a gluten free diet a deterioration of the morphology or a definite clinical relapse (repeated three times) on the reintroduction of gluten into the diet The frequency of so called transient gluten intolerance (normal mucosa on gluten containing diet for at least two years) (9) is also

PATIENTS

Malmo a city in South Sweden with about 260000 inhabitants (including 7% immigrants) has only one childrens hospital Children born and living in Malmo between 1 January 1966 and 31 December 1975 were included in the study 33405 infants were born alive in this period The patients ($n=49$) during 1967 to 1976 were admitted to the hospital from the outpatient clinic and from childrens welfare clinics attended by physicians from the children s hospital and by practitioner paediatricians Table 1 lists the main symptoms and signs

METHODS

Small intestinal biopsy was taken by a hydraulic capsule at the duodeno jejunal flexure under fluoroscopic control (17) On average two specimens were taken from this region on each occasion The biopsy specimen was oriented on a millipore filter and fixed in 4% formol solution with short after fixation in Bouin s solution After examination and photography in a dissecting microscopy the biopsy was serially cut into 5–6 μm sections Alternative slides were stained with haematoxylin and erythrosin with van Gieson stain and with periodic acid Schiff according to McManus The best oriented central cores of the specimens were used for assessment They were classified according to Alexander (1) as follows

Normal mucosa villous (finger and leaves) (Grade I) *slightly damaged mucosa* ridged (Grade II) *moderately damaged mucosa* convoluted (Grade III) *severely damaged mucosa* flat mosaic (Grade IV)

7 Ho P C & Lawton J W M Human colostral cells phagocytosis and killing of *E coli* and *C albicans* *J Pediatr* in press
8 Jelliffe E F P Maternal nutrition and lactation In *Breastfeeding and the mother* Ciba Foundation Symposium 45 North Holland Amsterdam 1976 p 119
9 Lascelles A K Gurner B W & Coombs R R Some properties of human colostral cells *Aust J Exp Biol Med Sci* 47 349 1969
10 Lawton J W M & Shortridge K F Protective factors in human breast milk and colostrum *Lancet I* 253 1977
11 McClelland D B L personal communication
12 Mohr J A Leu R & Mabry W Colostral leucocytes *J Surg Oncol* 2 163 1970
13 Mohr J A The possible induction and/or acquisition of cellular hypersensitivity associated with ingestion of colostrum *J Pediatr* 82 1062 1973
14 Murillo G J & Goldman A S The cells of human colostrum II Synthesis of IgA and β1c *Pediatr Res* 4 71 1970
15 Ogra S S Weintraub D & Ogra P L Immunologic aspects of human colostrum and milk III Fate and absorption of cellular and subcellular components in the gastrointestinal tract of the newborn *J Immunol* 119 245 1977
16 Papanicolaou G N & Maddi F V Further observations on the behaviours of human endometrial cells in tissue culture *Am J Obstet Gynecol* 78 156 1959
17 Parmely M J Beer A E & Billingham R E In vitro studies on the T lymphocyte population of human milk *J Exp Med* 144 358 1976
18 Pitt J Barlow B & Heird W C Protection against experimental necrotizing enterocolitis by maternal milk I Role of milk leukocytes *Pediatr Res* 11 906 1977
19 Pittard W B Polmar S H & Fanaroff A A The breast milk macrophage a potential vehicle for immunoglobulin transport *J Reticuloendothel Soc* 22 597 1977
20 Smith C W & Goldman A S The cells of human colostrum I *In vitro* studies of morphology and functions *Pediatr Res* 2 103 1968
21 Smith C W Goldman A S & Yates R D Interactions of lymphocytes and macrophages from human colostrum electron microscopic studies of interacting lymphocyte *Exp Cell Res* 69 409 1971
22 Yam L T Li C Y & Crosby W H Cytochemical identification of monocytes and granulocytes *Am J Clin Pathol* 55 283 1971

Submitted April 10 1978
Accepted Oct 4 1978

(J W M L) Department of Pathology
University of Hong Kong
Hong Kong

Table 2 *Incidence of coeliac disease 1966–1975 in Malmo*

	No of live births	No of coeliac children
1966	3 815	5
1967	3 760	3
1968	3 516	
1969	3 [illegible]95	4
1970	3 416	0
1971	3 [illegible]63	8
197[illegible]	3 [illegible]80	4
1973	3 035	[illegible]
1974	2 919	[illegible]
1975	2 806	4
Total	33 405	34 (1 98[illegible])

23) Thus the true incidence of coeliac disease is difficult to determine Early studies (1950 to 1963) report an incidence of 1 8000 (England and Wales (6)) 1 4000 (Scotland (6)) between 1 2000 and 1 6000 (London (5)) 1 6500 (Sweden (4)) and 1 3700 (Sweden (8)) This contrasts with recent studies with reported incidence of 1 597 (west of Ireland (19)) 1 890 (Switzerland (22)) 1 1100 (Glasgow (15)) and 1 1850 (west of Scotland (13)) The found incidence in Malmö of 1 1000 in a well defined population agrees with these latter figures The incidence in London (5) in Switzerland (22) and in the west of Scotland (13) was estimated with the known incidence of pyloric stenosis and cystic fibrosis as a comparison Had this method been used in this study the calculated incidence in Malmo would be between 1 400 and 1 660 The difference in incidences between the early (1950 to 1963) and the recent studies is probably due to the diagnosis being proved by routine intestinal biopsies in the later ones Even slight forms of the disease are sent to the hospital and diagnosed by intestinal biopsy Supporting this are the facts that 70% of the children in Malmo were diagnosed at an age of less than one year and that the frequency of the disease has increased three times since 1967 when intestinal biopsy was introduced in the routine clinical work and performed because of clinical grounds (3)

There is a strong association between coeliac disease and the leucocyte antigen HLA B8 60–90% of the patients are HLA B8 positive against 16–35% of the control population (24) It is of interest to note that Ireland which has the highest reported incidence of coeliac disease also has the highest HLA B8 frequency (35%) in the population (18) whereas it is 26% in the population of South Sweden (7)

Six (12%) of the 49 children with a severely damaged mucosa at the first biopsy on a gluten containing diet who became free of symptoms on a gluten free diet did not have a permanent gluten intolerance (9) The existence of this so called transient gluten intolerance is well known (10 11 20 25 26) Packer et al (21) suggest that a normal biopsy after 3 to 4 months of gluten challenge excludes a diagnosis of coeliac disease but recommend as does the European Society for Pediatric Gastroenterology and Nutrition (9) a further biopsy after two years Accordingly it is possible that the three children with a normal mucosa after 5 9 14 months of gluten containing diet have a transient gluten intolerance However we need more experience of this condition before deciding the time for the definite biopsy The found frequency agrees with that previously reported (10) The initial symptoms are the same as those of coeliac children but the infants with transient gluten intolerance were on average younger at debut This agrees with the experience of Kuitunen et al (10) The nature of this condition is obscure It can be a true transient gluten intolerance it can be a gluten intolerance secondary to a mucosal damage caused by cow s milk (26) or by an infection (2) Finally it can not be excluded that some children have a permanent low grade gluten intolerance giving symptoms in early infancy and taking a longer time for a relapse later in life

In any case it is important to recognize this condition This and the existence of symptom free relapses on gluten challenge emphasize the importance of following up these children and of evaluating the effect of gluten challenge with small intestinal biopsy

Table 1 *Main symptoms and signs in children with coeliac disease transient gluten intolerance and unverified and miscellaneous diagnosis*

	Group I Coeliac disease	Group II Transient gluten tolerance	Group III Unverified and miscellaneous diagnosis
Failure to thrive	24/34	3/6	6/9
Diarrhoea	26/34	6/6	6/9
Obstipation	3/34	0/6	2/9
Vomiting	9/34	1/6	2/9
Abdominal distension	23/34	1/6	5/9
Rickets	1/34	0 6	0/9

5 children with coeliac disease had normal stools

The first biopsy was taken while the child had a gluten containing diet and the second biopsy after 6–12 months on a gluten free diet After 1 to 1 5 years on a gluten free diet the child was challenged with gluten Usually a normal diet was given but in some instances 3 g to 15 g of gluten was added per day to the gluten free diet The time for the third biopsy depended on the clinical symptoms and if no symptoms appeared the challenge biopsy was taken after 4–5 months on this regime If no morphological deterioration occurred further biopsies were taken up to two years or longer on a normal diet *Children with a definite clinical relapse within days after gluten challenge began had again a gluten free diet for a month or more* Gluten challenge was then repeated twice The challenge was regarded as positive if symptoms reappeared each time and it was not considered necessary to perform an intestinal biopsy

RESULTS

49 children had a severely damaged mucosa in all specimens taken at the first biopsy They were classified into three groups according to the results of dietary treatment and of the gluten challenge

Group I Coeliac disease 34 children fulfilled the diagnostic criteria for coeliac disease given above 11 children had clear clinical symptoms on each gluten challenge and had no challenge biopsy Of the remaining 23 13 had no clinical symptoms but all had a moderately to severely damaged mucosa at the challenge biopsy Table 2 gives the number of births number of coeliac children each year and the incidence of coeliac disease for the 10 year period The over all incidence was 1 982 or 1 02 promille Mean age at debut of symptoms was 6 9 months and at first biopsy 13 months 70% of the children were less than one year old at the first biopsy

Three children were siblings One boy had younger sister (born 1976) with coeliac disease Another boy had a coexisting cystic fibrosis One girl was the child of immigrant parents

Group II Transient gluten intolerance Six of the 49 children (12%) did not relapse on gluten challenge and had a normal mucosa or slightly damaged mucosa on a normal diet for two years or more (2 2 2 5 2 5 4 and years) They had symptoms at 3 8 months old The mean age at first biopsy was 7 5 months *Initially all became symptomfree on a gluten free but cow s milk containing diet* The second biopsy on this dietary treatment showed *definite improvement of mucosal morphology*

Group III Unverified and miscellaneous diagnosis Two had a probable coeliac disease but do not yet fulfill the criteria Three had a normal mucosa in the challenge biopsy after 5 9 and 14 months of normal diet On clinical grounds two had a probable cow s milk protein intolerance a gluten challenge biopsy was not performed One boy had a multiple protein intolerance (gluten cow s milk egg and fish) *One child was lost from the follow up*

DISCUSSION

Family studies of coeliac disease have revealed that asymptomatic cases occur (12 14

Acta Pædiatr Scand 68 401–404 1979

MULTIPLE PITUITARY HORMONE DEFICIENCIES IN EIGHT SIBLINGS OF ONE JEWISH MOROCCAN FAMILY

M ADLER BIER A PERTZELAN Z LARON E LIEBERMAN[1] and S MOSES[1]

From the Institute of Pediatric and Adolescent Endocrinology Beilinson Medical Center Petah Tikva Sackler School of Medicine Tel Aviv University and the Division of Pediatrics Soroka Medical Center Faculty of Health Sciences Ben Gurion University of the Negev Beer Sheba Israel

ABSTRACT Adler Bier M Pertzelan A Laron Z Lieberman E and Moses S (Institute of Pediatric and Adolescent Endocrinology Beilinson Medical Center Petah Tikva Sackler School of Medicine Tel Aviv University and Division of Pediatrics Soroka Medical Center Faculty of Health Sciences Ben Gurion University of the Negev Beer Sheba Israel) Multiple pituitary hormone deficiencies in eight siblings of one Jewich Moroccan family Acta Paediatr Scand 68 401 1979 In a Jewish Moroccan inbred family 8 of 12 siblings were found to have multiple deficiencies of pituitary hormones including GH TSH and gonadotrophins The parents showed no deficiency and are in good health as are the other 4 siblings The investigations carried out indicate that in this family the etiology is hereditary in nature probably being autosomal recessive with the defect located in the pituitary gland

KEY WORDS Pituitary hormones panhypopituitarism genetic markers

Deficiency of several pituitary hormones including growth hormone is usually found in sporadic cases often being due to organic disease (9) The familial occurrence of panhypopituitarism which has been described as having an autosomal recessive mode of inheritance (13) is rare

Hereby presented is a family in which 8 of 12 siblings were found to be affected by hypopituitarism of varying degrees of expression

FAMILY HISTORY

This family came to our attention upon the referral of female siblings nos 4 and 5 because of marked growth retardation They were found to suffer from multiple pituitary hormone deficiencies and questioning of the parents revealed that others of the siblings were also small in stature and furthermore that consanguinal marriages had been frequent throughout the family s history It was therefore decided to investigate the whole family

The grandparents of Moroccan origin are first cousins (Fig 1) Both parents were in good health and reported that there were no known familial diseases nor any cases of excessively short stature There are 12 offsprings the 10th and 11th being twins All pregnancies and deliveries were uneventful and birth weights were normal although close to the lower limit

METHOD OF INVESTIGATION

Every family member with the exception of the 12th child who was born after cmopletion of this investigation underwent a thorough clinical examination which in

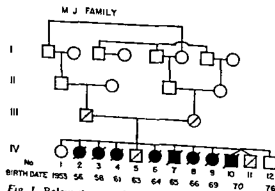


Fig 1 Relevant parts of the M J family tree as reported by the parents ■● pituitary insufficiency ⊠⊘ underwent endocrine and genetic markers studies

[1] Established Investigator of the Chief Scientist s Bureau of Health

ACKNOWLEDGEMENTS

Grants from the Swedish Medical Research Council (project no 5143 and 5364) Swedish Nutrition Foundation and Semper Nutrition Foundation supported this work

REFERENCES

1 Alexander J O Dermatitis herpetiformis *In* A Rook (ed) *Major problems in dermatology* vol 4 Saunders Co Ltd London Philadelphia and Toronoto 1975 p 236
2 Barnes G L & Townley R R W Duodenal mucosal damage in 31 infants with gastroenteritis *Arch Dis Child 48* 343 1973
3 Berg N O Borulf S Jakobsson I & Lindberg T How to approach the child suspected of malabsorption *Acta Paediatr Scand 67* 403 1978
4 Borgfors N & Selander P The incidence of coeliac disease in Sweden *Acta Paediatr Scand 57* 260 1968
5 Carter C Sheldon W & Walker C The inheritance of coeliac disease *Am Human Genet 23* 266 1959
6 Davidson L S P & Fountain J R Incidence of the sprue syndrome with some observations on the natural history *Br Med J 1* 1157 1950
7 Eberhard G Franzén G & Löw B Shizophrenia susceptibility and HL A antigen *Neuropsychology 1* 211 1975
8 Ek B Studies on idiopathic non tropical sprue *Acta Med Scand* Suppl 508 1970
9 European Society for Paediatric Gastroenterology Diagnostic criteria in coeliac disease *Acta Paediatr Scand 59* 461 1970
10 Kuitunen P Pelkonen P Perkkio M Savilahti E & Visakorpi J K Transient gluten intolerance *Acta Paediatr Belg 30* 250 1977
11 Lindberg T & Meuwisse G Transient coeliac disease—does it exist? *Acta Paediatr Scand* Suppl 236 p 56 1973
12 McCarthy C F Mylotte M Stevens F Egan Mitchell B Fottrell P F & McNicholl B Family studies on coeliac disease in Ireland *In* W Th J M Hekkens & A S Pena (eds) *Coeliac Disease Proc 2nd Int Coeliac Symposium* H E Stenfert Kroese Leiden 1974 p 311
13 McCrae W M Inheritance of coeliac disease *J Med Genet 6* 129 1969
14 MacDonald W C Dobbins W O & Rubin C I Studies of the familial nature of celiac sprue us biopsy of the small intestine *N Engl J Med 277* 4 1965
15 McNeish A S & Anderson C M Coeliac dise in childhood *Clin Gastroenterol 3* 127 1974
16 McNeish A S Rolles C J & Arthur L J I Criteria for diagnosis of temporary gluten intoleran *Arch Dis Child 51* 275 1976
17 Meeuwisse G W Lindberg T & Blomberg M hydraulic multiple intestinal biopsy apparatus for fants *Acta Paediatr Scand 63* 839 1974
18 Murtagh T J Reen D J & Gerally J HLA and B8 in coeliac disease *1st Int Symposium HLA and disease Paris* Abstract p 163 1976
19 Mylotte M Egan Mitchell B McCarthy C McNicholl B Incidence of coeliac disease in West of Ireland *Br Med J 1* 703 1973
20 Nusslé D Bozic C Cox J Delèze G Roul M & Mégevand A Non coeliac gluten intoler in infancy *3rd Int Coeliac Symposium* Galway 19
21 Packer S M Charlton V Keeling J W Risd R A Ogilvie D Rowlatt R J Larcher V F Harris J T Gluten challenge in treated coeliac d ease *Arch Dis Child 53* 449 1978
22 Shmerling D H Leisinger P & Prader A On familial occurrence of coeliac disease *Acta Paedi Scand 61* 501 1972
23 Stokes P L Asquith P & Cooke W T Genet of coeliac disease *Clin Gastroenterol 2* 547 1973
24 Strober W Abnormalities of the HLA system a gastrointestinal disease *In* J Dausset & A Svejga (eds) *HLA and disease* Munksgaard Copenhag 1977 p 168
25 Walker Smith J Transient gluten intolerance *Ar Dis Child 45* 523 1970
26 Visakorpi J K & Immonen P Intolerance to cow milk and wheat gluten in the primary malabsorpti syndrome in infancy *Acta Paediatr Scand 56* 1967

Submitted Sept 19 1978
Accepted Nov 3 1978

(T L) Department of Paediatrics
Malmo General Hospital
S 214 01 Malmo
Sweden

Acta Pædiatr Scand 68 401–404 1979

MULTIPLE PITUITARY HORMONE DEFICIENCIES IN EIGHT SIBLINGS OF ONE JEWISH MOROCCAN FAMILY

M ADLER BIER A PERTZELAN Z LARON E LIEBERMAN[1] and S MOSES[2]

From the Institute of Ped atric and Adolescent Endocrinology Beilinson Medical Center Petah Tikva Sackler School of Medicine Tel Aviv University and the [2]Division of Pediatrics Soroka Medical Center Faculty of Health Sciences Ben Gurion University of the Negev Beer Sheba Israel

ABSTRACT Adler Bier M Pertzelan A Laron Z Lieberman E and Moses S (Institute of Pediatric and Adolescent Endocrinology Beilinson Medical Center Petah Tikva Sackler School of Medicine Tel Aviv University and Division of Pediatrics Soroka Medical Center Faculty of Health Sciences Ben Gurion University of the Negev Beer Sheba Israel) Multiple pituitary hormone deficiencies in eight siblings of one Jewich Moroccan family Acta Paediatr Scand 68 401 1979 In a Jewish Moroccan inbred family 8 of 12 siblings were found to have multiple deficiencies of pituitary hormones including GH TSH and gonadotrophins The parents showed no deficiency and are in good health as are the other 4 siblings The investigations carried out indicate that in this family the etiology is hereditary in nature probably being autosomal recessive with the defect located in the pituitary gland

KEY WORDS Pituitary hormones panhypopituitarism genetic markers

Deficiency of several pituitary hormones including growth hormone is usually found in sporadic cases often being due to organic disease (9) The familial occurrence of panhypopituitarism which has been described as having an autosomal recessive mode of inheritance (13) is rare

Hereby presented is a family in which 8 of 12 siblings were found to be affected by hypopituitarism of varying degrees of expression

FAMILY HISTORY

This family came to our attention upon the referral of female siblings nos 4 and 5 because of marked growth retardation They were found to suffer from multiple pituitary hormone deficiencies and questioning of the parents revealed that others of the siblings were also small in stature and furthermore that consanguinal marriages had been frequent throughout the family s history It was therefore decided to investigate the whole family

The grandparents of Moroccan origin are first cousins (Fig 1) Both parents were in good health and reported that there were no known familial diseases nor any cases of excessively short stature There are 1? offsprings the 10th and 11th being twins All pregnancies and deliveries were uneventful and birth weights were normal although close to the lower limit

METHOD OF INVESTIGATION

Every family member with the exception of the 1?th child who was born after cmopletion of this investigation underwent a thorough clinical examination which in

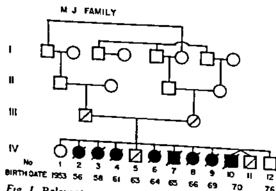


Fig 1 Relevant parts of the M J family tree as reported by the parents ■● pituitary insufficiency ⊠⊘ underwent endocrine and genetic markers studies

[1] Established Investigator of the Chief Scientist s Bureau Ministry of Health

Table 1 *Pertinent clinical and endocrinological data of family M J*
CA=Chronological age BA=Bone age FBS=Fasting blood sugar TRH=i v one bolus injection 100 µg/m LH RH i v one bolus injection 50 µg/m² ITT=i v one bolus insulin injection 0 1 u/kg

Patient at examination								Serum	
No	Sex	CA (yrs mos)	BA (yrs mos)	Height (cm)	S D	FBS (mg/dl)	hGH (ng/ml)	PBI (µg/dl)	T_4 (µg/dl)
1	Female	18.0	Mature	153.8	−1.3			4.8	
2	Female	15.1	10.0	137.5	−3.8	65	2.8	7.8	5.3
3	Female	9.4	2.9	100.5	−5.2	84	<2	3.0	
4	Female	6.4	2.6	92.2	−5.1	78	<2	2.8	
5	Male	12.9	13.0	152.0	Median	70	20.0		4.6
6	Female	10.10	7.0	119.3	−3.5	66	<2		1.[illegible]
7	Male	9.8	6.0	114.5	−3.3	64	<2		4.[illegible]
8	Female	8.7	7.0	109.6	−3.7	70	2.0		[illegible].8
9	Female	6.0	3.0	98.5	−3.1	70	<2		3.0
10	Male	5.4	3.0	96.5	−2.9	66	3.7		4.0
11	Male	5.4	4.6	112.0	Median	70	20.0		6.1
	Father	49.0		169.0	−0.8	102	>70		5.3
	Mother	38.0		153.0	−1.4	94	14.3		6.8

[a] hGH peak either after stimulation by insulin or arginine or at sleep (3) [b] Response after repeated i m injections of LH RH 100 µg/day for 5 days [c] Treated with T_4 [d] Serum levels went further down during hGH therapy

cluded measurements of body weight and height X rays of skull and wrist electroencephalography examination of eye fundi and routine testing of urine and blood

In order to detect a possible linkage between hypopituitarism and genetic markers blood samples were obtained from the parents and ten of their children (nos 2–11) Test for 18 different genetic loci (6 blood groups 10 redcell enzymes and 2 serum groups) were carried out

Hypothalamic pituitary hormone function was investigated using the following tests specific gravity of the urine and osmolality of the serum and urine (in all) *insulin tolerance test (in parents and patients nos 2–11)* arginine infusion test (in sibs nos 2 3 4 10 11) and sleep growth hormone secretion (4) (in no 11) serum PBI or T_4 and TRH test (6) (in parents and patients nos 2 3 4 6 7 8 9 10) and an LH RH test (3) (in parents and patients nos 2–11) It is of note that the TRH test was performed in most subjects prior to the institution of thyroxine treatment and in patients 3 and 4 after discontinuation of treatment which had been given for more than 10 years for a period of 3 weeks Furthermore the evaluation of *hGH secretion was performed only after a* euthyroid state had been achieved

RESULTS

The pertinent clinical and laboratory data of the children and parents are presented in Table 1 The parents and children nos 1 5 11 and 12 (the latter according to the parents) are of normal height and show normal development *Child no 6 is mentally retarded* The oldest daughter (no 1) is married and has two normal children Eight children (nos 2 3 4 6 7 8 9 10) were found to have a retardation of growth and skeletal maturation Girls nos 2 3 and 4 who have reached *pubertal* age show no signs of spontaneous puberty

In all of the subjects except no 12 who was not investigated X rays showed the skull including the sella turcica to be normal EEG *and eye fundi examinations as well as routine* urine and blood tests were all within normal limits The genetic marker studies were unrevealing In 12 systems all individual were found to be of the same type and hence these *were regarded as non informative In the other* 6 potentially informative systems none of the marker alleles was exclusively characteristic to affected or to non affected family members thus a close linkage between the hypopi*tuitarism with any of these systems was ruled* out

The results of the endocrine evaluation can be seen in Table 1

l)

lasma peak response									
RH		LH RH			ITT	Function of anterior pituitary axes			
SH ιU/ml)	Prolact (ng/ml)	At age (yrs mos)	LH (mU/ml)	FSH (mU/ml)	110HCS (μg/dl)	hGH	Thyroid	Gonadal	Adrenal
							Normal	Normal	
0 8	8 0	18 9	1 5	2 ?	43 0	Deficient	Deficient	Deficient	Normal
3 9	5 5	18 9	0 7[b]	1 3[b]	38 5	Deficient	Deficient	Deficient	Normal
5	5 0	15 10	0 5[b]	0 9[b]	?0 5	Deficient	Deficient	Deficient	Normal
		1? 9	9 6	? 6	19 7	Normal	Normal	Normal	Normal
7 7	15 0	1? 9	0 3	0 5	15 ?	Deficient	Deficient	Deficient	Normal
6 8	10 0	11 7	0 7	0 8	?3 5	Deficient	Deficient	Deficient	Normal
4 1	10 0	10 6	0 8	0 6	?4 5	Deficient	Deficient	Deficient	Normal
0 0	11 0	7 10	1 0	0 6	28 0	Deficient	Deficient	Deficient	Normal
0 7		5 4	0 5	0 5	40 0	Deficient	Deficient	Deficient	Normal
		5 4	5 4	1 7	17 5	Normal	Normal	Normal	Normal
	?7 0		16 7	9 6		Normal	Normal	Normal	
	37 0		0 3	?? 2		Normal	Normal	Normal	

Posterior pituitary

All the subjects tested had normal urine and plasma osmolalities

Anterior pituitary

Adrenal axis All the children tested showed a normal response of the plasma 110HCS to insulin hypoglycaemia

Growth hormone secretion thyroid axis and gonadal axis The parents and children nos 5 and 11 showed normal functions whereas off springs nos 2 3 4 6 7 8 9 and 10 showed a deficiency of all these hormones In chil dren nos 3 and 4 the plasma LH and FSH did not rise after repeated i m injections of LH RH (100 ug/day for 5 days)

Prolactin The parents had a normal basal level of plasma prolactin and a normal re sponse to TRH Among the children studied the basal level and response to TRH were within low normal limits in nos 2 6 7 8 and 9 In siblings 3 and 4 the response of prolactin to TRH was below normal

DISCUSSION

Several cases of familial occurrence of pan hypopituitarism have been reported in the lit erature The hormone deficiency most fre quently associated with that of growth hor mone is gonadotrophin deficiency followed in order by TSH deficiency and then by partial ACTH deficiency There is however a varia bility in the association of these hormone de ficiencies in any one family and between dif ferent families (12) Hooft & Casneuf (8) de scribed two brothers with hypopituitary dwarf ism but in both these cases there had been a prolonged breech delivery Ferrier & Stone (5) reported pituitary dwarfism associated with a very small sella turcica located in a sphenoid bone of abonormal morphology in two sisters Parks et al (10) described two sisters and one brother with hypopituitarism who had a large sella turcica Sadeghi Nejad & Senior (14) re viewed the syndrome with genetic aplasia of the anterior pituitary In all these cases an autosomal recessive mode of inheritance was suggested Two other reports have suggested

Table 1 *Pertinent clinical and endocrinological data of family M J*
CA=Chronological age BA=Bone age FBS=Fasting blood sugar TRH=i v one bolus injection 100 μg/m² LH RH i v one bolus injection 50 μg/m² ITT=i v one bolus insulin injection 0 1 u/kg

Patient at examination								Serum	
No	Sex	CA (yrs mos)	BA (yrs mos)	Height (cm)	S D	FBS (mg/dl)	hGH (ng/ml)	PBI (μg/dl)	T_4 (μg/dl)
1	Female	18 0	Mature	153 8	−1 3			4 8	
2	Female	15 1	10 0	137 5	−3 8	65	2 8	7 8	5 3
3	Female	9 4	2 9	100 5	−5 2	84	<2	3 0	
4	Female	6 4	2 6	92 2	−5 1	78	<2	2 8	
5	Male	12 9	13 0	152 0	Median	70	20 0		4 6
6	Female	10 10	7 0	119 3	−3 5	66	<2		1 [illegible]
7	Male	9 8	6 0	114 5	−3 3	64	<2		4 [illegible]
8	Female	8 7	7 0	109 6	−3 7	70	2 0		[illegible] 8
9	Female	6 0	3 0	98 5	−3 1	70	<2		3 0
10	Male	5 4	3 0	96 5	−2 9	66	3 7		4 0
11	Male	5 4	4 6	112 0	Median	70	20 0		6 1
	Father	49 0		169 0	−0 8	102	>20		5 3
	Mother	38 0		153 0	−1 4	94	14 3		6 8

hGH peak either after stimulation by insulin or arginine or at sleep (3) [a] Response after repeated i m injections of LH RH 100 μg/day for 5 days [c] *Treated with T_4* [d] *Serum levels went further down during hGH therapy*

cluded measurements of body weight and height X rays of skull and wrist electroencephalography examination of eye fundi and routine testing of urine and blood

In order to detect a possible linkage between hypopituitarism and genetic markers blood samples were obtained from the parents and ten of their children (nos 2–11) Test for 18 different genetic loci (6 blood groups 10 redcell enzymes and 2 serum groups) were carried out

Hypothalamic pituitary hormone function was investigated using the following tests specific gravity of the urine and osmolality of the serum and urine (in all) insulin tolerance test (in parents and patients nos 2–11) arginine infusion test (in sibs nos 2 3 4 10 11) and sleep growth hormone secretion (4) (in no 11) serum PBI or T_4 and TRH test (6) (in parents and patients nos 2 3 4 6 7 8 9 10) and an LH RH test (3) (in parents and patients nos 2–11) It is of note that the TRH test was performed in most subjects prior to the institution of thyroxine treatment and in patients 3 and 4 after discontinuation of treatment which had been given for more than 10 years for a period of 3 weeks Furthermore the evaluation of hGH secretion was performed only after a euthyroid state had been achieved

RESULTS

The pertinent clinical and laboratory data of the children and parents are presented in Table 1 The parents and children nos 1 5 11 and 12 (the latter according to the parents) are of normal height and show normal development Child no 6 is mentally retarded The oldest daughter (no 1) is married and has two normal children Eight children (nos 2 3 4 6 7 8 9 10) were found to have a retardation of growth and skeletal maturation Girls nos 2 3 and 4 who have reached pubertal age show no signs of spontaneous puberty

In all of the subjects except no 12 who was not investigated X rays showed the skull including the sella turcica to be normal EEG and eye fundi examinations as well as routine urine and blood tests were all within normal limits The genetic marker studies were unrevealing In 12 systems all individual were found to be of the same type and hence these were regarded as non informative In the other 6 potentially informative systems none of the marker alleles was exclusively characteristic to affected or to non affected family members thus a close linkage between the hypopituitarism with any of these systems was ruled out

The results of the endocrine evaluation can be seen in Table 1

EFFECT OF LONG TERM GH ADMINISTRATION ON PITUITARY THYROID FUNCTION IN IDIOPATHIC HYPOPITUITARISM

E CACCIARI A CICOGNANI P PIRAZZOLI F BERNARDI F ZAPPULLA
S SALARDI L MAZZANTI A BIASINI and E VALENTI

From the Department of Paediatrics University of Bologna Bologna Italy

ABSTRACT Cacciari E Cicognani A Pirazzoli P Bernardi F Zappulla F Salardi S Mazzanti L Biasini A and Valenti E (Department of Paediatrics University of Bologna Bologna Italy) Effect of long term GH administration on pituitary thyroid function in idiopathic hypopituitarism Acta Paediatr Scand 68 405 1979 —Twenty four euthyroid children with idiopathic pituitary dwarfism were studied The euthyroid state for seven of these patients was determined by negative physical examinations normal plasma T_4 assays and normal ^{13} I uptakes For the other children thyroid function was evaluated with T_3 and T assays and on the basis of the TRH test Each of the children was treated with HGH in one of three different ways The first group (five cases) was given a HGH dose ranging from 12 4 to 17 2 IU/m /week The second and third groups (nine and ten cases respectively) were treated with 10 and 20 IU/m²/week respectively Treatment was carried out for periods ranging from 6 months to 6 years After no less than 6 months of treatment and at intervals of 6 months (or some multiple of 6 months) plasma T and T assays as well as a TRH test were performed in each patient In some patients one of the indices was once beyond the upper or lower limit of the normal range (none of the children presented simultaneous abnormal levels of more than one index during the controls) This value however returned to within normal limits at the following control There was no correlation between T T and TSH with the duration of *HGH* therapy There was no significant difference between the groups of children treated with the different HGH doses These data seem to demonstrate that the risk of inducing an alteration in thyroid function in hypopituitary patients during HGH treatment is very slight and that the irregularly abnormal thyroid indices observed in some of the children during one of the controls might be an expression of their metabolic status at that moment

KEY WORDS Hypopituitarism HGH therapy thyroid function

In idiopathic hypopituitarism it has been demonstrated that the administration of human growth hormone (HGH) can induce alterations in thyroid function (7 11 13 14 15) In fact during HGH therapy a decrease in thyroid uptake (13) a reduction in plasma thyroid hormones (7) and a change in pituitary TSH reserve (7 11 14 15) have been observed This last was found to be lower in some cases (7 11 14) and higher in others (15) These contradictory results obtained from a limited number of patients and under experimental conditions that often are not comparable do not clearly answer the question whether there is a real risk of inducing hypothyroidism during HGH treatment or not

The purpose of the present study is to clarify this problem

MATERIALS AND METHODS

Twenty four prepubertal euthyroid children (15 males and 9 females) with idiopathic pituitary dwarfism were studied Their chronological age at the time of diagnosis ranged from 3 to 14 years The euthyroid state for 7 of these patients was confirmed by a negative physical examination normal plasma T assay and I uptake for the others it was confirmed by T_3 and T_4 determinations and by TSH values before and after TRH (thyrotrophin releasing hormone) administration (1)

Growth hormone function was evaluated by means of insulin induced hypoglycemia (0 1 U/kg i v) and arginine infusion (0 5 g/kg/30 min) (7) Growth hormone was measured utilizing the method of Molinatti et al (8) For receiving GH therapy a patient had to have a peak serum

a possible X linked recessive form of hypopituitarism (11, 15)

In the family presented here we were able to demonstrate deficiency of growth hormone secretion and of the hormones of the pituitary thyroid and pituitary gonadal axes Our finding in 2 children that LH and FSH did not rise after repeated stimulation of the LH RH together with the relatively low response of TSH and prolactin to TRH may point to a primary defect in the pituitary as suggested by Parks et al (10)

Since this disorder is a familial disease it is interesting to speculate as to whether the etiologic factor is a defect in a common hormone precursor or releaser which raises the possibility that a single gene may control more than one pituitary hormone or the etiology may be linked to an anatomic lesion being of the same origin as the syndrome of isolated aplasia of the anterior pituitary (1 14) but of a minor extent This could explain the variations in phenotypic expression observed in the various patients The high incidence of consanguinous marriage in this family and the number of siblings of both sexes affected can only point to a hereditary process of recessive nature

A recent review on the prevalence of pituitary dwarfism in Israel (2) revealed that the family reported here is the only family in Israel with MPHD (Multiple pituitary hormone deficiency and panhypopituitarism)

ACKNOWLEDGEMENT

We would like to thank Sarah Ashbel from the Dept of Human Genetics Sackler School of Medicine Tel Aviv University for her laboratory determinations of the genetic markers to Prof A Adam and Dr B Bonne for helpful suggestions

REFERENCES

1 Blizzard R M & Alberts M Hypopituitarism hypoadrenalism and hypogonadism in the newborn infant *J Pediatr 48* 782 1956
2 Adam A Josefsberg Z Pertzelan A Zadik Z Chemke J & Laron Z *The prevalence of four types of pituitary dwarfism in Israel J Med Genet* Submitted
3 Dickerman Z Prager Lewin R & Laron Z The effect of repeated injections of synthetic LH RH on *the response of plasma LH and FSH in young hypogonadotrophic hypogonadal patients Fertil Steril* 27 167 1976
4 Eastman C J & Lazarus L Growth hormone release during sleep in growth retarded children *Arch Dis Child 48* 502 1973
5 Ferrier P E & Stone E F Jr Familial pituitary dwarfism associated with an abnormal sella turcica *Pediatrics 43* 858 1969
6 Foley T P Jr Jacobs L S Hoffman W Daughaday W H & Blizzard R M Human prolactin and thyrotropin concentration in the serum of normal and hypopituitary children before and after the administration of synthetic thyrotropin releasing hormone *J Clin Invest 51* 2143 1972
7 Greulich W W & Pyle S I *Radiographic atlas of skeletal development of the hand and wrist* Stanford University Press Stanford California 1959 2nd ed
8 Hooft C & Casneuf J Familial hypopituitary dwarfism *Helv Paediatr Acta 21* 653 1966
9 Laron Z The hypothalamus and pituitary gland (hypophysis) *In* D Hubble (ed) *Paediatric endocrinology* Blackwell Sci Publ Oxford 1969 pp 35–111
10 Parks J S Tenore A Bongiovanni A M & Kirkland R T Familial hypopituitarism with large sella turcica *New Engl J Med 298* 698 1978
11 Phalan P D Connelley J Martin F I R & Wettenhall H N B X linked recessive hypopituitarism *In* D Bergsma (ed) *Clinical delineation of birth defects* X *The endocrine system birth defects* Orig Art Ser vol VII no 6 The Williams and Wilkins Co Fourth National Foundation—March of Dimes *Baltimore 1971* p 24
12 Rimoin D L Merimee T J Rabinowitz D & McKusick V A Genetic aspects of clinical endocrinology *Recent Progress Hormone Res 24* 365 1968
13 Rimoin D L & Schimke R N *Genetic disorders* of isolated aplasia of the anterior pituitary Diagnostic studies and treatment in the neonatal period
14 *Sadeghi Nejad A & Senior B A familial syndrome* of isolated aplasia of the anterior pituitary Diagnostic studies and treatment in the neonatal period *J Pediatr 84* 79 1974
15 Schimke R N Spaulding J J & Hollowell J F X linked congenital panhypopituitarism *In* D Bergsma (ed) *Clinical delineation of birth defects* X *The endocrine system birth defects* Orig Art Ser vol VII no 6 The Williams and Wilkins Co Fourth National Foundation—March of Dimes Baltimore 1971 p 21
16 Tanner J M Whitehouse R H & Takaishi M Standards from birth to maturity for height weight height velocity and weight velocity British children *Arch Dis Child 41* 454 and 613 1966

Submitted Aug 1 1978
Accepted Nov 13 1978

(Z L) Inst of Pediatric & Adolescent Endocrinology
Beilinson Medical Center
Petah Tikva Israel

Table 2 *T_3 (ng/ml) and T_4 (μg/100 ml) mean values ± S D before and after HGH therapy*

	Before therapy		After 6 months		After 1? months		After 18 months	
	T_3	T	T_3	T_4	T_3	T_4	T_3	T_4
x	1 6?	8 05	1 58	6 66*	1 25	7 65	1 73	7 60
S D	0 66	2 14	0 45	1 78	0 33	1 45	0 61	2 16
Range	0 7–? 6	3 9–11 7	1 1–2 7	4–8 9	0 8–1 8	5 5–8 8	0 8–2 7	4 8–10 5
No	17	24	9	10	6	6	7	7
	After 2 years		After ?½ years		After 3 years		After 3½ years	
	T_3	T	T_3	T	T_3	T	T_3	T
x	1 78	8 46	1 60	8 93	1 78	8 48	1 90	8 61
S D	0 8	? 27	0 42	1 34	0 46	? 49	0 61	2 01
Range	1–4 ?	3 5–14	1–? 4	7 5–1? 1	0 9–2 6	5 3–14	1 2–? 5	6 1–12 4
No	12	13	10	10	14	14	7	7
	After 4 years		After 5 years		After 6 years		Controls	
	T	T	T_3	T	T_3	T_4	T_3	T
x	1 80	8 15	10	8 93	1 8	6 7	1 78	8 93
S D	0 ?8	1 48	0 69	2 45	–	–	0 45	2 24
Range	1 4–?	6 6–9 7	1 3–2 5	6 5–11 4	–	–	0 8–2 7	4 ?–14
No	4	4	3	3	1	1	140	217

, $p<0 05$ paired *t* test for the difference with the values before therapy
* $p<0 01$ Student s *t* test for the difference with the controls
$p<0 05$ Student s *t* test for the difference with the controls

for T_3 in 3 cases for T_4 in 5 cases for basal TSH in 3 cases for TSH area in 5 cases for TSH peak and in 7 cases for TSH maximum increase During the following control however the altered value always returned to normal

3) The paired *t* test carried out for all the parameters at the various controls showed a T_3 increase at the 6th month ($p<0 05$) a decrease in TSH area after TRH at the 12th month ($p<0 01$) a decrease in basal TSH ($p<0 05$) and in TSH increase after TRH ($p<0 01$) at the 24th month

4) The mean value of the indices of pituitary thyroid function before therapy did not differ from that obtained after HGH treatment (Tables 2 and 3)

5) Whereas mean T_4 at the 6th month ($p<0 01$) and mean T_3 at the 12th month ($p<0 05$) were lower maximum TSH increase after TRH appeared to be at the 24th month slightly higher than the control mean value

6) The percentage of patients having a delayed maximum TSH peak (i e occurring at the 60th minute) did not change significantly during the treatment and was never significantly different from the percentage found in normal children (7 5%)

7) There was no correlation between the T_3 T_4 and TSH levels with the length of HGH therapy

8) There were no significant differences among the three groups of patients treated with different HGH dosages The differences for the various indices were evaluated comparing the mean values obtained before the treatment and at the various controls

DISCUSSION

A series of alterations in thyroid function linked to the presence of abnormal levels of growth hormone has been reported In acromegaly both hyperfunction and hypofunction (4 9) and more recently a lower pituitary response to the TRH stimulus (5 6) have been described It has been observed that administration of HGH in patients with GH deficiency

Table 1 *Clinical data of 24 patients with idiopathic hypopituitarism*

Case	Sex	Chronological age	Associated deficiencies	HGH treatment (IU/m²/week)	Years of therapy
1 G M	♂	9 5	ACTH	17 2	5
2 P L	♂	13 5	ACTH LH FSH	12 4	4
3 V P	♀	10 6	–	13 5	3½
4 V C	♂	11 3	LH FSH	16 8	6
5 B A	♂	14 0	LH FSH	16	½
6 L A	♂	10 4	–	10	2
7 L D	♂	5 5	–	10	3
8 C D	♂	3 2	–	10	2
9 C P P	♂	10 3	–	10	2
10 A V	♀	11 0	–	10	3½
11 B A	♀	11 0	–	10	1
12 F M	♂	11 9	–	10	1
13 P P	♂	10 6	–	10	½
14 D M V	♂	13 2	–	10	½
15 G M	♀	13 3	–	20	3½
16 M A	♀	9 0	–	20	3
17 G S	♀	6 5	–	20	3½
18 B C	♀	7 4	–	20	3½
19 M S	♂	10 1	–	20	3
20 B F	♂	13 5	–	20	3
21 T S	♀	4 6	–	20	2½
22 F G	♂	5 0	–	20	2½
23 C S	♂	13 7	–	20	1
24 T S	♀	5 7	–	20	1½

growth hormone concentration ≤4 ng/ml in response to both tests

LH FSH and ACTH pituitary reserves were investigated by means of an LH RH test (50 μg i v) and an insulin induced hypoglycemia test (0 1 U/kg i v) (2) LH and FSH were assayed according to the method of Reuter et al (12) Plasma ACTH was measured with the method of Vague et al (17) Two children (nos 1 and 2) had an associated ACTH deficiency and others (nos 2 4 and 5) had an associated LH FSH deficiency A subject was considered as having an LH FSH deficiency if during the LH RH test the values of both gonadotrophins were never above the sensitivity of the method (0 5 mIU/ml)

In all patients HGH (Grorm Serono) was administered i m as follows Group I (nos 1–5) 4 IU every other day for a total ranging from 12 4 to 17 2 IU/m²/week Group II (nos 6–14) 10 IU/m²/week Group III (nos 15–24) 20 IU/m²/week In the last two groups the HGH dose was divided into three injections per week The treatment was carried out for periods ranging from six months to six years During the observation period none of the patients received corticosteroid or gonadotrophin therapy In all the children regardless of the period of treatment there was an increase in the growth rate of at least 2 5 cm/year as compared to the year preceding treatment After no less than six months of treatment and at intervals of six months (or multiples of six months) all the children underwent plasma T_3 and T_4 determinations and a TRH test performed as previously described (1) with a 100 μg dose of TRH rapidly injected i v and samples collection at 0 20 and 60 min The TSH pituitary reserve was studied by evaluating the peak the maximum increase and the area under the curve after TRH administration (3) Since a delayed TSH response to TRH may be an expression of a thyroid hypofunction of hypothalamic nature (5) the percentage of children with the TSH peak at the last sample (60 min) was evaluated both before and during HGH treatment TSH was evaluated according to the method of Odell et al (10) using the MRC standard 68/38 Plasma T_4 was determined at first by a competitive protein binding analysis (Thiopac 4 Amersham) and more recently by radioimmunoassay (T_4 Riakit Biodata) Plasma T_3 was evaluated by radioimmunoassay (T_3 Ria kit Biodata)

The statistical analysis of the results was carried out using Student s *t* test the paired *t* test and chi square as well as by calculating the correlation coefficient *r*

RESULTS

A summary of the clinical and laboratory data is shown in Tables 1 2 and 3 The results can be summarized as follows

1) None of the 24 subjects examined presented more than one abnormal index at one control

2) Values above or below the normal range during one of the controls were seen in 1 case

3) The duration of treatment after the first six months does not seem to influence the levels of the classical parameters of thyroid function In fact T_3 T_4 and TSH before and after TRH show no correlation with the duration of the treatment

4) The HGH dosage does not influence the effect of therapy on pituitary thyroid function In fact no differences were seen among the three groups of patients treated differently

5) The temporary irregularly abnormal variations in one of the thyroid indices observed in some of the patients during treatment may be an expression of the metabolic situation in the patient at that moment As a matter of fact the evaluation of pituitary thyroid indices in a normal child on different days may give completely different results (our unpublished data)

ACKNOWLEDGEMENTS

We wish to thank in particular Dr M Sesso (Hoechst AG) for the generous supply of TRH Dr M Capelli (Central Laboratory S Orsola Hospital) for technical assistance

REFERENCES

1 Cacciari E Bernardi F Salardi S Tassoni P Cicognani A Pirazzoli P Zappulla F & Zingoni S The thyrotropin releasing hormone test in idiopathic pituitary dwarfism *Helv Paediatr Acta* 29 481 1974

2 Cacciari E Cicognani A Tassoni P Pirazzoli P Bernardi F Salardi S & Zappulla F Problemi e risultati nella diagnosi e nella terapia delle iposomie con deficit dell ormone dell accrescimento *Minerva Pediatr* 26 1709 1974

3 Cacciari E Cicognani A Pirazzoli P Tassoni P Zappulla F Salardi S & Bernardi F Relationships among the secretion of ACTH GH and cortisol during the insulin induced hypoglycemia test in the normal and obese child *J Clin Endocrinol Metab* 40 80 1975

4 Codaccioni J L Jaquet P Bismuth J & Freychet P La thyroide dans l acromégalie *Ann Endocrinol* 3 68 1971

5 Faglia G Beck Peccoz P Ferrari C Ambrosi B Spada A Travaglini P & Paracchi S Plasma thyrotropin response to TRH in patients with pituitary and hypothalamic disorders *J Clin Endocrinol Metab* 37 595 1973

6 Kastin A J Shally A V Gonzales Barcena D Schalch D S & Lee L Different responses to two hypothalamic hormones in acromegalics *Arch Intern Med* 130 9?3 197?

7 Lippe B M Van Herle A J Lanfranchi S H Uller R P Lavin N & Kaplan S A Reversible hypothyroidism in growth hormone deficient children treated with human growth hormone *J Clin Endocrinol Metab* 40 612 1975

8 Molinatti G M Massara F Pennisi F Scassellati G A Strumia B & Vancheri L Radioimmunoassay of growth hormone *J Nucl Biol Med* 13 76 1969

9 Muktar E Wilkinson R Alexander L Appleton D & Hall R Thyroid function in acromegaly *Lancet* II ?79 1971

10 Odell W D Wilber J F & Paul W E Radioimmunoassay of thyrotropin in human serum *J Clin Endocrinol Metab* 25 1179 1965

11 Porter B A Refetoff S Rosenfield R L De Groot L J Fang V S & Stark V Abnormal thyroxine metabolism in hyposomatotrophic dwarfism and inhibition of responsiveness to TRH during GH therapy *Pediatrics* 51 668 1973

12 Reuter A M Hendrick J C & Franchimont P Mise au point d un dosage radioimmunologique rapide des gonadotrophines *Ann Biol Clin* 31 479 1973

13 Root A W Bongiovanni A M & Eberlein W R Inhibition of thyroidal radioiodine uptake by human growth hormone *J Pediatr* 76 4?2 1970

14 Root A W Snyder P J Rezvani I Di George A M & Utiger R D Inhibition of thyrotropin releasing hormone mediated secretion of thyrotropin by human growth hormone *J Clin Endocrinol Metab* 36 103 1973

15 Sato T Suzuki Y Taketani T Ishiguro K Masuyama T Takata I Sano M Kawashima H Koizumi D & Nakajima H Enhanced peripheral conversion of thyroxine to triiodothyronine during HGH therapy in GH deficient children *J Clin Endocrinol Metab* 45 3 4 1977

16 Snyder P J & Utiger R D Inhibition of thyrotropin releasing hormone by small quantities of thyroid hormones *J Clin Invest* 51 2077 197?

17 Vague P Oliver C Jaquet P & Vague J Le dosage de l'ACTH plasmatique Résultats chez les sujets normaux *Rev Eur Etud Clin Biol* 16 485 1971

18 Vigneri R Squatrito S Pezzino V Filetti S & Polosa P The effect of short term triiodothyronine administration on thyroxine response to exogenous TSH in man *J Clin Endocrinol Metab* 41 974 1975

Submitted June 29 1978
Accepted Oct 21 1978

(E C) Department of Paediatrics
Via Massarenti 11
40138 Bologna
Italy

Table 3 *Mean values ± S D of basal peak area and maximum TSH (μU/ml) increase during the TRH test (100 μg i v)*

	Basal value	Peak	Area	Increment	Basal value	Peak	Area	Increment
	Before HGH therapy (n=17)				After 6 months of therapy (n=10)			
x	5 76	16 70	39 79	10 94	3 24	13 53	31 96	10 79
S D	4 51	7 93	21 65	6 38	2 37	4 89	11 79	4 58
Range	0 8–14	6 9–43 5	15 4–114	4 2–30 1	0 8–8 6	4 6–20 5	11 6–52	3 8–17 8
	After 12 months of therapy (n=6)				After 18 months of therapy (n=7)			
x	4 45	13 80	30 63[b]	9 35	4 84	13 47	37 86	8 53
S D	1 89	4 48	11 67	4 86	3 49	4 25	10 48	4 98
Range	2 5–8	9 1–20	21 5–51	4 4–15 7	0 8–10 2	8 5–18 8	20 7–47	2 2–13 6
	After 2 years of therapy (n=13)				After 2½ years of therapy (n=10)			
x	3 54	20 09[a]	46 96	16 55	3 96	18 64	40 45	14 69
S D	1 60	10 71	23 72	10 36	2 91	8 82	19 21	7 84
Range	1 2–7 7	8 2–40	20 6–92	4 6–37 3	0 6–9	3 2–33 6	6 7–75	2 7–25 8
	After 3 years of therapy (n=14)				After 3½ years of therapy (n=7)			
x	4 62	15 79	37 06	11 13	6 19	16 53	41 54	10 44
S D	2 46	7 35	17 68	6 63	4 26	10 42	25 95	11 16
Range	0 8–10 2	7 4–34	19 8–82	2 8–29 6	0 8–14 6	2 5–36 5	5 6–91 6	0 6–32 8
	After 4 years of therapy (n=4)				After 5 years of therapy (n=3)			
x	6 45	16 95	36 23	9 75	6 23	12 20	30 80	5 97
S D	4 41	6 38	13 86	4 61	5 89	7 93	23 36	2 34
Range	2 6–12	11 2–26	29–57 1	3 2–14	2 2–13	5 6–21	14–57 5	3 4–8
	After 6 years of therapy (n=1)				Controls (n=80)			
x	1 6	6 7	16 9	5 1	4 64	16 27	38 00	11 63
S D	–	–	–	–	2 53	7 09	15 83	6 40
Range	–	–	–	–	0 6–10 2	5 5–36 8	11 5–86	3 2–30 5

[a] $p<0\ 05$ paired *t* test for the difference with the values before treatment
[b] $p<0\ 01$ paired *t* test for the difference with the values before treatment

may diminish ^{131}I uptake by the thyroid (13) may lower plasma level of the total T_4 free T_4 and T_3 (7) or on the contrary may increase plasma T_3 level with a normal T_4 level (14) Porter et al (11) found that the decreased TSH response to TRH after a few days of HGH therapy became normal again after some months of treatment These data contrast with the results obtained by Lippe et al (7) who found (in 3 out of 6 patients) a decrease in the response to TRH after 6 months of treatment Sato et al (15) found an increase in the response to TRH after 3–12 months of therapy These contradictory results may be due to the low number of cases studied to the different parameters taken into consideration and to the different experimental conditions

Our investigation carried out on a substantial number of subjects treated with different HGH doses and for periods of time ranging from six months to six years seems to demonstrate the following

1) The risk of inducing a significant alteration of thyroid function in the growth hormone deficient patient by HGH treatment is evidently very slight

2) If some alterations in the thyroid function are induced by the HGH administration these are most likely to occur during the first months of therapy i e during the period we did not investigate and they must be reversible This is also demonstrated by the fact that during the first three months of HGH therapy a faster conversion of T_4 into T_3 is reported (15) this can lead to a decrease in T_4 (15) in ^{131}I uptake (18) and in TSH response to TRH (16)

3) The duration of treatment after the first six months does not seem to influence the levels of the classical parameters of thyroid function In fact T_3, T_4 and TSH before and after TRH show no correlation with the duration of the treatment

4) The HGH dosage does not influence the effect of therapy on pituitary thyroid function In fact no differences were seen among the three groups of patients treated differently

5) The temporary irregularly abnormal variations in one of the thyroid indices observed in some of the patients during treatment may be an expression of the metabolic situation in the patient at that moment As a matter of fact the evaluation of pituitary thyroid indices in a normal child on different days may give completely different results (our unpublished data)

ACKNOWLEDGEMENTS

We wish to thank in particular Dr M Sesso (Hoechst AG) for the generous supply of TRH Dr M Capelli (Central Laboratory S Orsola Hospital) for technical assistance

REFERENCES

1 Cacciari E Bernardi F Salardi S Tassoni P Cicognani A Pirazzoli P Zappulla F & Zingoni S The thyrotropin releasing hormone test in idiopathic pituitary dwarfism Helv Paed at Acta 29 481 1974

Cacciari E Ci ognani A Tassoni P Pirazzoli P Bernardi F Salardi S & Zappulla F Problemi e risultati nella diagnosi e nella terapia delle iposomie con deficit dell ormone dell accrescimento Minerva Ped at 26 1 09 1974

3 Cacciari E Cicognani A Pirazzoli P Tassoni P Zappulla F Salardi S & Bernardi F Relationships among the secretion of ACTH GH and cortisol during the insulin induced hypoglycemia test in the normal and obese child J Clin Endocrinol Metab 40 80 1975

4 Codaccioni J L Jaquet P Bismuth J & Freychet P La thyroide dans l acromégalie Ann Endocrinol 32 768 1971

5 Faglia G Beck Peccoz P Ferrari C Ambrosi B Spada A Travaglini P & Paracchi S Plasma thyrotropin response to TRH in patients with pituitary and hypothalamic disorders J Clin End crinol Metab 37 595 1973

6 Kastin A J Shally A V Gonzales Barcena D Schalch D S & Lee L Different responses to two hypothalamic hormones in acromegalics Arch Intern Med 130 973 1972

7 Lippe B M Van Herle A J Lanfranchi S H Uller R P Lavin N & Kaplan S A Reversible hypothyroidism in growth hormone deficient children treated with human growth hormone J Clin Endocrinol Metab 40 612 1975

8 Molinatti G M Massara F Pennisi F Scassellati G A Strumia B & Vancheri L Radioimmunoassay of growth hormone J Nucl Biol Med 13 76 1969

9 Muktar E Wilkinson R Alexander L Appleton D & Hall R Thyroid function in acromegaly Lancet II 279 1971

10 Odell W D Wilber J F & Paul W E Radioimmunoassay of thyrotropin in human serum J Clin Endocrinol Metab 25 1179 1965

11 Porter B A Refetoff S Rosenfield R L De Groot L J Fang V S & Stark V Abnormal thyroxine metabolism in hyposomatotrophic dwarfism and inhibition of responsiveness to TRH during GH therapy Pediatrics 51 668 1973

12 Reuter A M Hendrick J C & Franchimont P Mise au point d un dosage radioimmunologique rapide des gonadotrophines Ann Biol Clin 31 479 1973

13 Root A W Bongiovanni A M & Eberlein W R Inhibition of thyroidal radioiodine uptake by human growth hormone J Pediatr 76 42 1970

14 Root A W Snyder P J Rezvani I Di George A M & Utiger R D Inhibition of thyrotropin releasing hormone mediated secretion of thyrotropin by human growth hormone J Clin Endocrinol Metab 36 103 1973

15 Sato T Suzuki Y Taketani T Ishiguro K Masuyama T Takata I Sano M Kawashima H Koizumi D & Nakajima H Enhanced peripheral conversion of thyroxine to triiodothyronine during HGH therapy in GH deficient children J Clin Endocrinol Metab 45 324 1977

16 Snyder P J & Utiger R D Inhibition of thyrotropin releasing hormone by small quantities of thyroid hormones J Clin Invest 51 2077 1972

17 Vague P Oliver C Jaquet P & Vague J Le dosage de l ACTH plasmatique Résultats chez les sujets normaux Rev Eur Etud Clin Biol 16 485 1971

18 Vigneri R Squatrito S Pezzino V Filetti S & Polosa P The effect of short term triiodothyronine administration on thyroxine response to exogenous TSH in man J Clin Endocrinol Metab 41 974 1975

Submitted June 29 1978
Accepted Oct 21 1978

(E C) Department of Paediatrics
Via Massarenti 11
40138 Bologna
Italy

Acta Pædiatr Scand 68 411–418 1979

CHANGES IN SERUM CONCENTRATIONS OF THYROID HORMONES AND THYROID HORMONE BINDING PROTEINS DURING EARLY INFANCY

Studies in Healthy Fullterm Small for gestational Age and Preterm Infants Aged 7 to 240 Days

B BROCK JACOBSEN and L HUMMER

From the University Clinic of Paediatrics Children s Hospital Fuglebakken and the Department of Nuclear Medicine Rigshospitalet Copenhagen Denmark

ABSTRACT Jacobsen B B and Hummer L (University Clinic of Paediatrics Children s Hospital Fuglebakken and the Department of Nuclear Medicine Rigshospitalet Copen hagen Denmark) Changes in serum concentrations of thyroid hormones and thyroid hormone binding proteins during early infancy Studies in healthy fullterm small for gestational age and preterm infants aged 7 to 240 days Acta Paediatr Scand 68 411 1979 — Serum concentrations of thyrotropin (TSH) thyroxine (T_4) triiodothyronine (T_3) thyroxine binding globulin (TBG) prealbumin (TBPA) and albumin (Alb) were determined in 492 blood samples from 127 fullterm (FT) 91 small for-gestational age (SGA) and 88 preterm (PT) healthy infants aged 7 to 240 days *Serum T* decreased about 20% during the first month of life In infants aged 7–49 days serum T concentrations were significantly lower in SGA than in FT infants and even lower values were found in PT infants *Serum T_3* increased 50–70% reaching maximal values by 50–79 days of life Serum T_3 levels were higher in FT than in SGA infants throughout the observation period In PT infants serum T_3 increased from low values to levels which exceeded those of SGA and FT infants by 120–240 days of life *Serum TSH* level did not change with age and was ≤5 mU/l in all infants *Serum TBG* values were high compared to normal adult values and did not change significantly with age Com parable serum TBG values were found in FT SGA and PT infants *Serum TBPA* increased with age Serum TBPA increased gradually in FT infants In SGA infants serum TBPA increased from low values to levels which by 120–240 days of life exceeded those of PT and FT infants In PT infants a decrease in serum TBPA appeared before the rise commenced *Serum Alb* increased gradually in FT SGA and PT infants during the observation period Serum Alb in PT infants aged 30–119 days was lower than those in FT infants with similar ages These physiological changes in serum concentrations of thyroid hormones and hormone binding proteins during early infancy should be considered when interpreting thyroid func tion tests in infants with various maturity

KEY WORDS Thyroxine triiodothyronine thyrotropin thyroxine binding globulin pre albumin albumin infants

During the first week of life pronounced changes in serum concentrations of thyrotro pin (TSH) and thyroid hormones occur (9 16) Later in infancy and childhood serum TSH remains constant at the level of normal adults (1 3 11) whereas serum protein bound iodine and thyroxine (T_4) show a progressive de crease with age (5 6 11 22 24) Serum tri iodothyronine (T_3) levels are increased in in fants aged 1–6 months compared with cord blood levels (1 8 14 18 21) but the postnatal alterations have not been completely eluci dated Very little is known about the thyroid hormone binding protein concentrations in in fancy A higher binding capacity of thyroxine binding globulin (TBG) and a lower binding capacity of thyroxine binding prealbumin (TBPA) in newborn infants compared with those in older children and adults have been reported (7 21)

Table 2 *Median and range of serum concentrations of th\roxine and triiodoth)ronine in full term (FT) small for gestational age (SGA) and preterm (PT) infants with age ranging from 7-240 da\s*

(*n*)=number of blood samples *Range* indicates lowest and highest values

	Postnatal age in days					
	7–13	14– 9	30–49	50–79	80–119	1?0–?40
Se um T_4 (nmol/l)						
FT						
Median	17	149	143	1 6	143	131
Range	95– 83	85–?60	75–196	64–198	95–197	64–170
(*n*)	(6?)	(35)	(33)	(36)	(18)	(19)
SGA						
Median	165	138	1?6	1?9	128	108
Range	103–?70	88–?19	90–180	91–167	93–187	82–178
(*n*)	(33)	(37)	(25)	(19)	(11)	(17)
?T						
Median	143	1??	118	107	1?7	1 1
Range	8?–216	77–??1	?9–237	50–193	90–?11	82–148
(*n*)	(?4)	(4?)	(23)	(19)	(?1)	(1?)
	$p<0 01$	$p<0 01$	$p<0 01$	N S	N S	N S
Se um T_3 (nmol/l)						
FT						
Median	00	? 47	? 69	3 10	2 97	2 34
Range	1 16–3 49	1 67–5 0?	1 77–5 04	1 80–4 72	2 11–7 50	1 16–3 77
(*n*)	(34)	(18)	(19)	(19)	(10)	(19)
SGA						
Median	? 04	11	? 23	2 61	. 55	? 32
Range	1 3–? 91	1 ?8–3 11	1 44–3 49	1 0–3 33	1 87–3 54	1 47–3 18
(*n*)	(16)	(6)	(?1)	(17)	(1?)	(17)
PT						
Median	1 77	1 72	2 04	? 88	2 67	3 00
Range	1 ?9–? 85	0 66–3 00	0 30–3 35	1 03–3 88	1 88–3 89	2 16–3 67
(*n*)	(1?)	(?6)	(12)	(13)	(13)	(1?)
	$p<0 01$	$p<0 01$	$p<0 01$	$p<0 05$	$p<0 05$	$p<0 05$

p is the level of significance for the Kruskal Wallis test N S =not significant

Th\rotropin concentrations

Serum TSH concentrations remained essentially constant during infancy and were similar in FT SGA and PT infants (≤5 mU/l)

Th\roid hormone concentrations

Serum T_4 concentrations decreased during the first month of life in all maturity groups (Fig 1) The T_4 decrease was about 20% In infants aged 7 to 49 days serum T_4 levels were significantly lower in SGA than in FT infants and even lower values appeared in PT infants (Table 2) Later comparable serum T_4 levels were found in the three groups of infants

Serum T_3 concentrations *increased* to maximal values at 50–79 days of life (Fig 1) The T_3 increase was 50–70% The serum levels of T_3 in FT SGA and PT differed significantly throughout the study period (Table 2) From 7 to 49 days of life the lowest serum T_3 level appeared in PT infants but later serum T_3 level in PT exceeded those of SGA and FT infants

The ratios between serum T_4 and serum T_3 concentrations (T_4/T_3) decreased during the study period (from 85 to 45 (median values))

Table 1 *Body weights (g) in fullterm (FT) small for gestational age (SGA) and preterm (PT) infants*

Median and range values in each age group are presented range indicates lowest and highest values (*n*)=number of blood samples

	Postnatal age in days					
	7–13	14–29	30–49	50–79	80–119	120–240
FT						
Median	3 335	3 580	4 250	5 050	5 705	7 370
Range	2 590–4 250	2 660–4 400	3 000–6 500	3 550–7 670	4 350–8 900	5 650–9 700
(*n*)	(62)	(35)	(34)	(36)	(18)	(21)
SGA						
Median	2 420	2 745	3 250	3 780	4 805	6 590
Range	1 760–2 980	2 000–3 680	2 520–5 300	2 930–4 780	4 150–6 000	3 650–7 830
(*n*)	(33)	(38)	(25)	(20)	(12)	(17)
PT						
Median	2 240	2 415	2 820	3 440	4 470	6 050
Range	1 620–2 850	1 000–3 330	1 210–3 670	1 520–4 710	2 070–6 000	3 670–8 450
(*n*)	(24)	(42)	(23)	(19)	(21)	(17)
	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.01$	$p<0.05$

p is the level of significance for the Kruskal Wallis test

Recently we reported data indicating different serum levels of thyroid hormones and hormone binding proteins in fullterm (FT) small for gestational age (SGA) and preterm (PT) babies during the first week of life (16 17) The present report is intended to characterize serum concentrations of TSH thyroid hormones and thyroid hormone binding proteins in healthy babies later in infancy

MATERIALS AND METHODS

A total number of 306 euthyroid infants with ages ranging from 7 to 240 days were studied The infants were in good clinical condition without infections respiratory distress or treatment which could influence serum thyroid hormone levels Gestational age and maturity were assessed as reported previously (15) The fullterm infants group consisted of 127 infants with gestational ages from 37–42 weeks and birth weights 2 750–4 350 g The SGA infant group consisted of 91 infants with gestational ages from 37–41 weeks and birth weights 1 790–2 750 g Finally 88 infants were preterm infants with gestational ages from 25–36 weeks and birth weights from 830–2 750 g

A total number of 492 blood samples were collected i e 1 to 6 samples per infant Blood was drawn for reasons other than clinically suspected thyroid dysfunction Informed parental consent was obtained Blood was drawn from a peripheral vein and serum frozen at −20 C until analyses were performed Blood sampling was not complete for determination of all thyroid variables only serum T_4 concentrations were measured in nearly all infants (see Tables) Usually ≥10 determinations of the variables were obtained in each age group

Serum concentrations of TSH and T_3 were measured by radioimmunoassay and serum T_4 was determined by competitive binding technique (15) Serum concentrations of TBG TBPA and albumin (Alb) were determined using rocket immunoelectrophoresis as reported previously (17) The protein concentrations were presented in terms of those obtained in a reference serum (arbitrarily set to 100 units per liter) The absolute serum TBG TBPA and Alb concentrations in the reference serum are 93 mg/l 297 mg/l and 43 000 mg/l respectively (17)

In the *statistical* calculations nonparametric tests were used (25) The Kruskal Wallis test was used to decide whether the differences between thyroid variables in FT SGA and PT infants signify population differences or represent merely chance variations The Wilcoxon test was used to determine whether variables in two groups of infants were different The null hypothesis was rejected at the 0 05 level of significance

RESULTS

Body weights

Table 1 shows median and range values of body weights in each age group The body weights of FT SGA and PT infants differed significantly throughout the study period The median increase in body weight was 120% in FT and 170% in low birth weight infants

Table 3 *Median and range of serum concentrations of thyroxine binding proteins (TBG TBPA Albumin) in fullterm (FT) small for gestational age (SGA) and preterm (PT) infants*

(n)=number of blood samples *Range* indicates lowest and highest values

	Postnatal age in days					
	7-13	14-29	30-49	50-79	80-119	1 0-740
Serum TBG (arb u /l)						
FT						
Median	163	190	188	195	191	198
Range	1?1-187	135- 19	91-268	112-.96	138-285	133-268
(n)	(15)	(10)	(13)	(19)	(11)	(14)
SGA						
Median	169	167	167	164	170	145
Range	1?5-?17	90-??0	130-?07	116-219	111-218	108-211
(n)	(11)	(19)	(?0)	(15)	(10)	(1?)
PT						
Median	170	166	179	155	199	186
Range	1 1-? 0	1 0-?10	100-251	76-?30	96-?67	1?7-255
(n)	(1?)	(?0)	(10)	(15)	(1)	(10)
	N S	N S	N S	N S	N S	p<0 0?
Serum TBPA (arb u /l)						
FT						
Median	37	44	44	51	48	5?
Range	14-46	17-51	2?-61	4-73	?1-70	27-78
(n)	(13)	(7)	(11)	(14)	(8)	(15)
SGA						
Median	27	29	34	43	54	75
Range	19-7\	15-53	17-68	?0-59	33-70	?3-104
(n)	(9)	(1)	(16)	(13)	(9)	(11)
PT						
Median	30	8	21	39	49	59
Range	19-41	18-47	1 -3?	16-6?	34-61	26-88
(n)	(9)	(17)	(7)	(14)	(9)	(10)
	N S	N S	p<0 01	p<0 0?	N S	N S
Serum Alb (arb u /l)						
FT						
Median	8	9?	9	9?	104	99
Range	66-94	71-97	85-166	76-1?7	83-145	73-110
(n)	(14)	(8)	(13)	(14)	(11)	(13)
SGA						
Median	77	84	89	92	96	97
Range	69-96	63-104	76-105	79-109	89-1?0	67-111
(n)	(11)	(19)	(18)	(14)	(10)	(1?)
PT						
Median	85	80	84	88	9?	97
Range	55-106	63-96	4 -105	4?-95	48-10	8?-103
(n)	(1)	(18)	(10)	(14)	(8)	(9)
	N S	N S	p<0 01	N S	N S	N S

N S = not significant

cantly lower than those in FT infants with similar ages ($p<0.05$) Only in age group 30-49 days serum Alb in SGA infants differed significantly from those in FT and PT babies

DISCUSSION

The present data indicate that pronounced changes in serum concentrations of thyroid hormones and hormone binding proteins oc

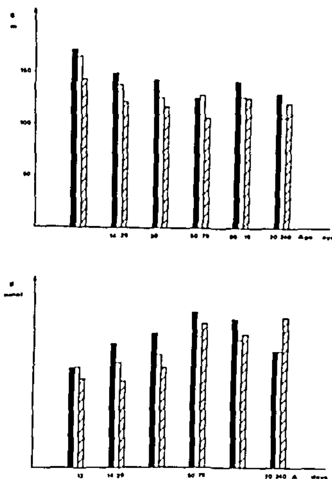


Fig 1 Median values of serum thyroxine (T_4) and triiodothyronine (T_3) concentrations in fullterm (closed bars) small for gestational age (open bars) and preterm (hatched bars) infants ranging in age from 7 to 240 days

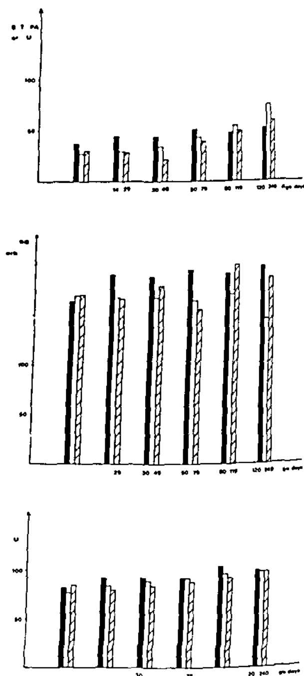


Fig 2 Median values of serum concentrations of thyroxine binding globulin (TBG) prealbumin (TBPA) and albumin (Alb) in fullterm (closed bars) small for gestational age (open bars) and preterm (hatched bars) in age from 7 to 240 days

Thyroid hormone binding protein concentrations (Fig 2)

Median and range values of serum TBG TBPA and Alb concentrations are shown in Table 3 The *p* values of the Kruskal Wallis test are also given A wide range in serum protein concentrations was seen in all age groups *Serum TBG* level did not change significantly with age but great variations were seen particularly in PT infants The serum TBG levels in FT SGA and PT infants did not differ significantly except in infants aged 120–240 days (Table 3) *Serum TBPA* increased with age (Table 3) the increases were more pronounced in low birth weight infants (100–175%) than in FT infants (40%) In SGA infants serum TBPA increased from low levels to levels exceeding those of FT infants at 120–240 days of life ($p<0.05$) In PT infants serum TBPA decreased initially before the TBPA rise commenced

Serum Alb concentrations in FT as well as in low birth weight infants presented a gradual increase (about 20–25%) (Table 3) Serum Alb in PT infants aged 30–119 days were signifi

TBG and TBPA concentrations associated with idiopathic respiratory distress syndrome (18)

During the first week of life serum concentrations of TBG TBPA and Alb differ significantly in FT SGA and PT infants (17) and the present data indicate that thyroid hormone binding protein levels also later in infancy are influenced by the maturity A tendency towards higher serum TBG levels in FT than in low birth weight newborns was seen Serum TBPA in SGA infants increased from very low levels to TBPA levels which exceeded those of FT infants aged 120–240 days (Fig 2) In PT babies a decrease in serum TBPA level appeared initially after which a pronounced increase occurred Serum albumin level tended to be higher in FT than in SGA and PT infants but the postnatal serum albumin increases were of the same magnitude in the three groups of infants similar findings in mature babies have been reported previously (20) These postnatal changes in serum protein concentrations probably reflect alterations and differences in synthesis and metabolism of TBG TBPA and Alb in FT SGA and PT infants Several factors influence the protein metabolism (23) but the mechanisms that determine these changes in early infancy as well as later in childhood (10 11) are unknown

Knowledge of these postnatal alterations in serum levels of thyroid hormones and hormone binding proteins seems to be of clinical significance The changes in serum T_4 and T_3 concentrations have to be considered in diagnosis and treatment of hypothyroidism in early infancy It is also important to keep the differences between FT SGA and PT infants in mind when interpreting the *in vitro* thyroid function tests in infants of various maturity

ACKNOWLEDGEMENTS

We are grateful for excellent technical assistance from the staff of the laboratories involved Thanks to J Nyboe cand act Rigshospitalet for great help with the statistical analyses The secretarial assistance of miss A Jacobsen is greatly appreciated

Human TSH for iodination and antihuman TSH were kindly donated by the National Pituitary Agency Bethesda USA The human TSH international reference preparation 68/38 was given by the Medical Research Council Holly Hill London

The study was supported by the Danish Medical Research Council and the Dagmar Marshall Foundation

REFERENCES

1 Abuid J M Klein A H Foley T P & Larsen P R Total and free triiodothyronine and thyroxine in early infancy *J Clin Endocrinol Metab 39* 263 1974

2 Bernal J & Refetoff S The action of thyroid hormone *Clin Endocrinol 6* 227 1977

3 Binet E Sizonenko P C & Job J C Résultats du dosage radioimmunologique de la thyreostimuline hypophysaire (TSH) dans le plasma chez l enfant normal et dans les hypothyreoides congenitales *Arch Fr Pediatr 28* 179 1971

4 Brien T G Fay J A & Griffin E A Thyroid status in the newborn infant Effective thyroxine ratio and free thyroxine index *Arch Dis Child 49* 225 1974

5 Chadd M A Davies D F & Gray O P Protein bound iodine in premature infants *Arch Dis Child 43* 217 1968

6 Danowski T S Johnston S Y Price W C McKelvy M Stevenson S S & McCluskey E R Protein bound iodine in infants from birth to one year of age *Pediatrics 7* 240 1951

7 De Nayer P Van Den Schrieck H G & De Visscher M Le transport serique de la thyroxine chez l enfant *Ann Endocrinol (Paris) 26* 360 1965

8 Dutau G Bayard F Bennet P & Rochiccioli P Dosage radioimmunologique de la triiodothyronine plasmique chez l enfant normal et dans differentes circonstances pathologiques *Arch Fr Pediatr 31* 861 1974

9 Erenberg A Phelps D L Lam R & Fisher D A Total and free thyroid hormone concentrations in the neonatal period *Pediatrics 53* 211 1974

10 Fisher D A & Dussault J H Development of the mammalian thyroid gland *In* M A Greer & D H Solomon (eds) *Handbook of physiology Endocrinology III Thyroid* American Physiological Society Washington 1974 p 21

11 Fisher D A Sack J Oddie T H Pekary A E Hershman J M Lam R W & Parslow M E Serum T TBG T uptake T reverse T and TSH concentrations in children 1 to 15 years of age *J Clin Endocrinol Metab 45* 191 1977

12 Hays G C & Mullard J E Normal free thyroxine index values in infancy and childhood *J Clin Endocrinol Metab 39* 958 1974

13 Hesch R D Gatz J Juppner H & Stubbe P TBG-dependency of age related variations of thyroxine and triiodothyronine *Horm Metab Res 9* 141 1977

14 Homoki J Loos U Rothenbuchner G Grimm V & Teller W M TSH T_4 T_3 and reverse T_3

cur in early infancy These changes are related to the postnatal age as well as to the maturity of the infants

Earlier observations of a decrease in total serum T_4 and protein bound iodine concentrations during infancy (1 4 5 6 12 19 21 22 24) were confirmed by the present study A gradual decrease in serum T_4 occurred during the first month of life in all groups of infants During the first week serum T_4 concentrations were lower in SGA than in FT infants and even lower values appeared in PT infants (16) The present study indicates that these significant differences continue until 30–49 days of age

Previous studies have demonstrated higher serum T_3 concentrations in infants aged 1–6 months than in newborns (1 8 14 21) but detailed informations of the postnatal changes were not given In the present study a gradual increase in total serum T_3 levels was found in FT and SGA infants from 7–13 days of age to 50–79 days of age after which a decrease in median values appeared in FT and SGA infants The maximal serum T_3 values were higher than serum T_3 levels of normal adults (normal range 1 10–2 40 nmol/l) The T_3 increase seemed to be more pronounced in FT than in SGA infants In PT infants the T_3 increase was *delayed* because serum T_3 values in PT infants aged 7–13 days and 14–29 days were similar in accordance with a recent report by Homoki et al (14) Later serum T_3 in PT infants increased and exceeded T_3 levels of SGA and FT infants by 120–240 days of life In that way serum T_3 concentrations in FT SGA and PT infants differed significantly throughout the study period

The results of present and previous studies (1 9 16) indicate a *diphasic* pattern in total serum T_3 concentrations during early infancy After the presumably TSH induced increase in free and total serum T_3 to peak values by 24–48 hours of life a decrease occurs Later serum T_3 again increases to maximal values at 50–79 days of age but in all groups of infants serum TSH concentrations remained ≤5 mU/l

Enhanced peripheral T_4 to T_3 conversion is suggested as one cause of the increase in serum T_3 contemporary with the serum T_4 decrease a conversion which may be different in FT SGA and PT infants However variations in *secretion* of T_3 and T_4 from the thyroid gland influenced by age and maturity may also be of significance Since most of the extrathyroidal pool of T_4 and T_3 is located in the intracellular compartment (2) the present data probably reflect pronounced postnatal changes in thyroid hormone metabolism In this connection it is noteworthy that the increase in body weight was comparable in SGA and PT infants and more pronounced than in FT babies

Serum TBG levels did not change significantly whereas serum TBPA and Alb increased in all groups of infants The decrease in serum T_4 was quantitatively more pronounced than the T_3 increase and the data indicate a *decrease in the saturation* of hormone binding sites upon the serum proteins during early infancy This could also explain some of the age related changes in T_3 resin uptake and other *in vitro* tests reported previously (4 17 19 22)

Very little information of serum levels of thyroid hormone binding proteins during infancy is available Measurement of serum T_4 binding capacity has shown high TBG and low TBPA binding capacities in infants in comparison with those in adults (7) Using radioimmunoassay Hesch et al (13) found increased serum TBG concentrations in infants and older children but the number of samples was rather small Montalvo et al (21) observed a decrease in serum TBG binding capacity between one week and one month of age in *mature* infants which was not confirmed by the present data However the median values of serum TBG concentrations tended to be lower in FT and SGA and higher in PT infants in comparison with the TBG values of healthy infants during the first week of life reported previously (17) Recently we reported pronounced laterations in serum

ta Pædiatr Scand 68 419–422 1979

PHARMACOKINETICS OF AMIKACIN IN INFANTS AND PRE SCHOOL CHILDREN

D A KAFETZIS C A SINANIOTIS C J PAPADATOS and J KOSMIDIS

From the Second Department of Paediatrics and the First Department of Propedeutic Medicine Athens University School of Medicine Athens Greece

ABSTRACT Kafetzis D A Sinaniotis C A Papadatos C J and Kosmidis J (Second Department of Paediatrics and First Department of Propedeutic Medicine Athens University School of Medicine Athens Greece) Pharmacokinetics of amikacin in infants and pre school children Acta Paediatr Scand 68 419 1979 —The pharmacokinetic properties of amikacin sulfate in infants and children aged from three weeks to 6 years were studied during treatment with doses of 7 5 mg/kg every 12 hours using standard assay methods and technique of two compartment open model kinetic analysis Peak serum concentrations of amikacin were measured 30 or 60 min after the first intramuscular injection These ranged from 11 8 μg/ml to 23 μg/ml in infants and from 9 0 μg/ml to 29 μg/ml in children Five minutes after the first intravenous bolous injection they varied from 16 μg/ml to 29 8 μg/ml in infants and from 34 μg/ml to 42 μg/ml in children Twelve hours after injection serum concentrations were less than 0 8 μg/ml in all patients Mean serum half lives of amikacin in infants and children were 2 1 hours and 2 0 hours after intramuscular and 2 2 and 2 0 hours after intravenous administration respectively No evidence of accumulation was observed after four days treatment The amount of antibiotic recovered within 12 hours from the urine in all patients ranged from 34 5 to 65 % of an intramuscular dose and from 45 8 to 63 3 % of an intravenous dose The dosage regime of 7 5 mg/kg body weight given every 12 hours should be safe and effective for the treatment of infections in the age groups studied

KEY WORDS Amikacin pharmacokinetics half life urinary excretion infants and pre-school children

During the last two years an increasing number of infections caused by gentamicin resistant gram negative bacteria occurred in the Aglaia Kyriakou Children s Hospital Athens Amikacin a new semisynthetic aminoglycoside antibiotic which is stable to most gentamicin inactivating enzymes has proved useful in the treatment of such infections Its use is therefore increasing in our hospital and since similar experience of increasing bacterial resistance to gentamicin has been reported by others (3) a more extensive usage of amikacin is generally expected The kinetics of amikacin have been extensively studied in adults and neonates (1 2 4) but there is little information about children aged from two weeks to 6 years (7 9) Infections in the latter age group are often due to gram negative bacteria (infected burns and accidental wounds peritonitis chest infections in cystic fibrosis compromised defense systems etc) and if gentamicin resistant are likely to be treated with amikacin

We have therefore undertaken a pharmacokinetic study of amikacin in infants and children aged from three weeks to 6 years

PATIENTS AND METHODS

Twenty four infants and children all inpatients at the Aglaia Kyriakou Children s Hospital were studied They all suffered from serious infections for which amikacin was indicated The diagnosis and the clinical response are shown in Table 1

These 4 patients were aged 20 days to 6 years There were 13 infants of a mean age of 3 5 months and 11 children of a mean age of 3 1 years The dosage of amikacin sulfate was 7.5 mg/kg body weight given every 12 hours

serum concentrations in the premature infant during the first month of life (abstract) *Acta Endocrinol (Kbh)* Suppl 215 1978 p 71
15 Jacobsen B B Andersen H J Dige Petersen H & Hummer L Pituitary thyroid responsiveness to thyrotropin releasing hormone in preterm and small for gestational age newborns *Acta Paediatr Scand* 66 541 1977
16 Jacobsen B B Andersen H J Peitersen B Dige Petersen H & Hummer L Serum levels of thyrotropin thyroxine and triiodothyronine in fullterm small for gestational age and preterm newborn babies *Acta Paediatr Scand* 66 681 1977
17 Jacobsen B B Peitersen B Andersen H J & Hummer L Serum concentrations of thyroxine binding globulin prealbumin and albumin in healthy fullterm small for gestational age and preterm newborn infants *Acta Paediatr Scand* 1979 accepted for publication
18 Jacobsen B B Peitersen B & Hummer L Serum concentrations of thyrotropin thyroid hormones and thyroid hormone binding proteins during acute and recovery stages of idiopathic respiratory distress syndrome *Acta Paediatr Scand* 68 257 1979
19 Koivisto M Simila S Haapalahti J & Reinila M Thyroid function assay using the effective thyroxine ratio in infancy *Scand J Clin Lab Invest* 33 195 1974
20 Krasilnikoff P A *Albumin metabolism in normal mature and premature children* FADL Copenhagen 1975
21 Montalvo J M Wahner H W Mayberry W E & Lum R K Serum triiodothyronine total thyroxine and thyroxine to triiodothyronine ratios in paired maternal-cord sera and at one week and one month of age *Pediatr Res* 7 706 1973
22 O Halloran M T & Webster H L Thyroid function assays in infants *J Pediatr* 91 916 1977
23 Robbins J Thyroxine binding proteins *In* G A Jamieson & T J Greenwalt (eds) *Progress in clinical and biological research* Elsevier Oxford 1976 p 331
24 Ryness J The measurement of serum thyroxine in children *J Clin Pathol* 25 726 1972
25 Siegel S *Nonparametric statistics for the behavioral sciences* McGraw Hill Kogakusha Tokyo 1956

Submitted July 6 1978
Accepted Oct 16 1978

(B B J) The University Clinic of Paediatrics
Children s Hospital Fuglebakken
Drosselvej 57
2000 Copenhagen F
Denmark

ta Pædiatr Scand 68 419–422 1979

PHARMACOKINETICS OF AMIKACIN IN INFANTS AND PRE SCHOOL CHILDREN

D A KAFETZIS C A SINANIOTIS C J PAPADATOS and J KOSMIDIS

From the Second Department of Paediatrics and the First Department of Propedeutic Medicine Athens University School of Medicine Athens Greece

ABSTRACT Kafetzis D A Sinaniotis C A Papadatos C J and Kosmidis J (Second Department of Paediatrics and First Department of Propedeutic Medicine Athens University School of Medicine Athens Greece) Pharmacokinetics of amikacin in infants and pre school children Acta Paediatr Scand 68 419 1979—The pharmacokinetic properties of amikacin sulfate in infants and children aged from three weeks to 6 years were studied during treatment with doses of 7.5 mg/kg every 12 hours using standard assay methods and technique of two compartment open model kinetic analysis Peak serum concentrations of amikacin were measured 30 or 60 min after the first intramuscular injection These ranged from 11 8 μg/ml to 23 μg/ml in infants and from 9 0 μg/ml to 29 μg/ml in children Five minutes after the first intravenous bolous injection they varied from 16 μg/ml to 29 8 μg/ml in infants and from 34 μg/ml to 42 μg/ml in children Twelve hours after injection serum concentrations were less than 0 8 μg/ml in all patients Mean serum half lives of amikacin in infants and children were 2 1 hours and 2 0 hours after intramuscular and 2 2 and 2 0 hours after intravenous administration respectively No evidence of accumulation was observed after four days treatment The amount of antibiotic recovered within 12 hours from the urine in all patients ranged from 34 5 to 65% of an intramuscular dose and from 45 8 to 63 3% of an intravenous dose The dosage regime of 7 5 mg/kg body weight given every 12 hours should be safe and effective for the treatment of infections in the age groups studied

KEY WORDS Amikacin pharmacokinetics half life urinary excretion infants and preschool children

During the last two years an increasing number of infections caused by gentamicin resistant gram negative bacteria occurred in the Aglaia Kyriakou Children s Hospital Athens Amikacin a new semisynthetic aminoglycoside antibiotic which is stable to most gentamicin inactivating enzymes has proved useful in the treatment of such infections Its use is therefore increasing in our hospital and since similar experience of increasing bacterial resistance to gentamicin has been reported by others (3) a more extensive usage of amikacin is generally expected The kinetics of amikacin have been extensively studied in adults and neonates (1 2 4) but there is little information about children aged from two weeks to 6 years (7 9) Infections in the latter age group are often due to gram negative bacteria (infected burns and accidental wounds peritonitis chest infections in cystic fibrosis compromised defense systems etc) and if gentamicin resistant are likely to be treated with amikacin

We have therefore undertaken a pharmacokinetic study of amikacin in infants and children aged from three weeks to 6 years

PATIENTS AND METHODS

Twenty four infants and children all inpatients at the Aglaia Kyriakou Children s Hospital were studied They all suffered from serious infections for which amikacin was indicated The diagnosis and the clinical response are shown in Table 1

These 24 patients were aged 20 days to 6 years There were 13 infants of a mean age of 3.5 months and 11 children of a mean age of 3 1 years The dosage of amikacin sulfate was 7 5 mg/kg body weight given every 12 hours

Table 1. *Clinical condition and response to treatment of the 24 patients studied*

M: male; F: female; I.M.: intramuscular; I.V.: intravenous

Diagnosis	No. of patients M	No. of patients F	Route of administ. I.M.	Route of administ. I.V.	Response to treatment: Cured	Improved	Failed
Urinary tract infection	4	5	9	–	8	1	–
Peritonitis	6	1	6	1	5	1	1
Infected burn	5	–	2	3	4	1	–
Septicemia	2	–	–	2	2	–	–
Pneumonia	1	–	1	–	1	–	–
Wound infection	1	–	1	–	1	–	–

The antibiotic was administered by intramuscular (i.m.) injection in 18 patients of whom 10 infants (7 male and 3 female) and 8 children (6 male and 2 female), and as bolus intravenous (i.v.) injection in 6: three infants (all male) and 3 children (2 male and one female). If patients required an additional antibiotic, amikacin was given alone for the first 12 hours of therapy and then the second drug was added. Blood samples were obtained by heel prick or venipuncture prior to, and at 1/2, 1, 2, 4, 6, 8 and 12 hours after the first dose. Urine was collected in plastic bags before and at 0–2, 2–4, 4–8 and 8–12 hours post dose. To detect possible accumulation of the drug, blood specimens were also obtained prior to the morning injection on the fourth treatment day.

Concentrations of amikacin were measured using an agar well plate technique with *bacillus subtilis* 1904 E as indicator organism. In blood samples from patients treated with amikacin plus either lincomycin or clindamycin a strain of *Klebsiella* was used as indicator organism, and for specimens from those receiving also penicillin G or ampicillin, penicillinase concentrate BBL 1:50 was added. Large plates were used and standards (ranging from 1.0 μg/ml to 20.0 μg/ml) as well as specimens, suitably diluted when necessary, were set in triplicate. Serum half life was determined by using a two compartment open model as follows: after the distribution phase was completed, the equation for the regression line of the log of serum antibiotic concentrations was calculated by the method of the least mean squares. The half life was determined by dividing the $\log_{10}2$ by the slope of the line.

RESULTS

Mean serum concentrations of amikacin in infants and children following the first i.m. or i.v. injection are shown in Table 2.

In 10 infants receiving i.m. injections peak serum concentrations were obtained 30 to 60 min post dose and ranged from 11.8 μg/ml to 23.0 μg/ml (mean 18.4±3.0). Four hours post dose levels averaged 4.2 μg/ml and at 8 hours

Table 2. *Mean serum concentration of amikacin in infants and pre-school children after i.m. or i.v. injection of 7.5 mg/kg*

Age group and route	No. of pat.	Serum concentration (μg/ml) at indicated times (minutes or hours after dose): 5	30	1	2	4	6
Infants i.m.	10		17±3.6 (11.8–23.0)	15.7±2.8 (9.0–19.5)	10.6±3.4 (3.2–16.4)	4.2±1.1 (1.7–5.9)	2.6±0.34 (2.[illegible]–3.[illegible])
Children i.m.	8		18.4±5.4 (8.0–29.0)	17.7±3.7 (9.0–22.0)	11.6±4.6 (2.8–17.5)	3.7±1.4 (1.5–5.9)	2.2±1.0 (0.8–3.7)
Infants i.v.	3	24.3±5.9 (16.0–29.8)	14.7±4.8 (7.9–18.4)	11.6±5.1 (4.4–16.2)	8.7±4.8 (2.4–14.0)	5.1±3.1 (1.2–8.9)	3.8[b]±0.4 (3.4–4.[illegible])
Children i.v.	3	37.3±3.4 (34.0–42.0)	19.8±3.2 (16.4–24.1)	13.1±2.3 (9.5–16.0)	7.8±0.5 (7.1–8.2)	4.0[b]±0.1 (3.9–4.2)	2.0±0.3 (1.6–[illegible].4)

Figures with a ± sign indicate standard deviation, in parenthesis are ranges.

[b] Only two specimens studied.

Table 3 *Mean urinary excretion of amikacin after i m or i v injection of 7 5 mg/kg in infants and children*

NUP no urine passed in this collection period

Age group and route	No of pat	Mean urinary concentration (μg/ml first column) and mean percentage recovered (second column) at indicated collection periods (hours) 0–2		2–4		4–8		8–12		0–12
Infants i m	6	61	16 3±12 4 (NUP–31 1)	336	14 3±8 7 (6 2–30 0)	152	6 6±2 6 (4 4–11 0)	31	1 8±0 8 (0 8–2 9)	39 0±5 1 (34 5–48 7)
Children i m	3	345	18 5±13 0 (NUP–28 8)	215	10 2±7 2 (NUP–15 8)	184	19 7±10 4 (6 2–31 5)	134	6 3±7 0 (NUP–16 2)	54 6±7 4 (47 7–65 0)
Infants i v	3	561	4 7±11 0 (9 1–32 8)	51	3±15 0 (9 3–43 3)	78	2 8±2 1 (NUP–5 3)	31	3 2±2 2 (1 1–6 4)	53 0±5 4 (45 8–58 9)
Children i v	3	6 4	24 0±1 8 (5 9–34 3)	490	20 4±6 4 (15 4– 9 5)	168	15 1±5 2 (10 8–2 5)	24	2 0±0 8 (0 8–2 9)	61 5±2 0 (58 7–63 2)

Figures with a ± sign indicate standard deviation in parenthesis are ranges

	12	Mean serum half life (hours)
5_0 36 8–2 1)	<0 8	15±0 17 (1 9– 40)
3_0 7 0 8–)	<0 8	0 ±0 24 (1 6–2 28)
4+0 8 0 8– 2)	<0 8	2 21±0 15 (0– 33)
0_0 2 8–1)	<0 8	04±0 (1 79–2 30)

1 5 μg/ml with a mean serum half life of 2 1 hours

In 3 infants treated with i v injections peak serum concentrations were measured 5 min post dose and ranged from 16 0 μg/ml to 29 8 μg/ml (mean 24 3±5 9) Thirty minutes after injection serum levels averaged 14 7 μg/ml at 4 hours 5 1 μg/ml and at 8 hours they averaged 1 4 μg/ml Mean serum half life was 2 2 hours in these patients

In 8 of the older children who received i m injections peak serum concentrations were achieved from 30 to 60 min after the dose and ranged from 9 0 μg/ml to 29 0 μg/ml (mean 19 3±5 1) Four hours after injection serum levels averaged 3 7 μg/ml and at 8 hours 1 3 μg/ml while serum half life averaged 2 0 hours

Finally in 3 children receiving i v injections peak serum levels 5 min after injection ranged from 34 0 μg/ml to 42 0 μg/ml (mean 37 3±3 4) Thirty minutes post dose mean serum levels were 19 8 μg/ml while at 4 hours levels averaged 4 0 μg/ml and at 8 hours 1 0 μg/ml Serum half life in these children averaged 2 0 hours

In all patients amikacin blood levels were undetectable (less than 0 8 μg/ml) 12 hours post dose No cumulation of the drug was observed on the fourth treatment day serum levels before the morning injection being undetectable in all but one patient a 5 month old girl in whom blood levels was 1 1 μg/ml

Excretion of the drug in urine of infants and children is shown in Table 3 In 6 infants 34 5% to 48 7% of the i m dose was recovered within 12 hours After i v injection in 3 infants 45 8% to 58 9% of the drug was recovered during the same period

In 3 children aged from one to 6 years recovery rate ranged from 47 7% to 65% of the i m dose and in another 3 children given i v injections it ranged from 58 7% to 63 2%

Urine levels of amikacin were much higher after i.v. injection ranging in the first four hours from 186 μg/ml to 750 μg/ml in infants and from 210 μg/ml to 806 μg/ml in children. After i.m. injection it ranged from 40 μg/ml to 950 μg/ml in infants and from 110 μg/ml to 495 μg/ml in children. Urine concentrations were bactericidal in all patient groups for the whole period of 12 hours after injection.

DISCUSSION

Pediatric drug therapy, and particularly calculation of dosage is complicated by the rapid change in body weight and body composition in children (8).

Studies with amikacin sulfate in adults have shown that the desirable peak serum concentrations are 15–35 μg/ml and 5.0 μg/ml or less at the time of the next dose (5). It has also been shown that the serum half life of aminoglycosides is approximately two hours in persons with normal renal function and excretion is mainly affected by glomerular filtration (2, 5).

This study, involving 24 seriously ill infants and children has shown that mean peak blood concentrations of amikacin, given intramuscularly or intravenously in a dose of 7.5 mg/kg every 12 hours were in the therapeutic and safe range for both age and routes of administration. On the other hand 8 hours following administration blood concentrations were already below the therapeutic levels for many pathogens. However, all patients included in the study were clinically and bacteriologically cured (Table 1) (6). Serum half life measured at the phase of elimination was found to be almost the same as that reported for adults (2, 5), but it was slightly longer in infants (about 2.2 hours) than in children (about 2.0 hours). Vogelstein and his colleagues (9), using a new pharmacokinetic technique, have reported somewhat shorter half lives (1.6 hours) in children aged from 4 to 15 years and reported no cumulation after four 8 hourly doses of 4?0 mg/m². Whether continuation of an 8 hourly regime would result in eventual cumulation and potential toxicity requires further investigation. However, at least for the age groups that we have investigated, that is 20 days to 6 years, such high dosage and frequent administration of amikacin may not be necessary in view of our satisfactory results with 7.5 mg/kg given every 12 hours (Table 1).

REFERENCES

1. Cabana, B. E. & Taggart, J. G.: Comparative pharmacokinetics of BBK8 and Kanamycin in dogs and humans. Antimicrob Agents Chemother 3: 478, 1973.
2. Clarke, J. T., Libke, R. D., Regamey, C. & Kirby, W. M.: Comparative pharmacokinetics of amikacin and kanamycin. Clin Pharmacol Ther 15: 610, 1974.
3. George, R. H. & Healing, D. E.: Gentamicin resistance. Lancet I: 258, 1976.
4. Howard, J. B., McCracken, G. H., Trujillo, H. & Mohs, E.: Amikacin in newborn infants: comparative pharmacology with kanamycin and clinical efficacy in 45 neonates with bacterial diseases. Antimicrob Agents Chemother 10: 205, 1976.
5. Jackson, G. G.: Present status of aminoglycoside antibiotics and their safe, effective use. Clin Therapeut 1: 200, 1977.
6. Kafetzis, D. A., Kosmidis, J. C., Sinaniotis, C. A. & Papadatos, C. J.: Therapeutic and kinetic studies with amikacin in infants and children. In W. Siegenthaler & R. Luthy (eds.) Current Chemotherapy. American Society for Microbiology, Washington, D.C. 1978, vol. 2, p. 1002.
7. Khan, A. J., Evans, H. E., Jhaveri, R., Chang, C. T. & Hochstein, L.: Amikacin pharmacokinetics in the therapy of childhood urinary tract infection. Pediatrics 58: 873, 1976.
8. Rane, A. & Wilson, J. T.: Clinical pharmacokinetics in infants and children. Clin Pharmacokin 1: 2, 1976.
9. Vogelstein, B., Kowarski, A. A. & Lietman, P. S.: The pharmacokinetics of amikacin in children. J Pediatr 91: 333, 1977.

Submitted Oct. 2, 1978
Accepted Nov. 2, 1978

(D. A. K.) 2nd Department of Paediatrics
Athens University
"Aglaia Kyriakou" Children's Hospital
Athens (609)
Greece

Acta Pædiatr Scand 68 423–430 1979

DOSAGE OF SALICYLATES FOR CHILDREN WITH JUVENILE RHEUMATOID ARTHRITIS

A Prospective Clinical Trial with Three Different Preparations of Acetylsalicylic Acid

ANNA LIISA MAKELA TORSTI YRJÁNA and MAURI MATTILA

From the Departments of Paediatrics and Clinical Chemistry University of Turku and the Department of Pharmacology University of Helsinki Finland

ABSTRACT Makela A L Yrjana T and Mattila M (Departments of Paediatrics and Clinical Chemistry University of Turku and Department of Pharmacology University of Helsinki Finland) Dosage of salicylates for children with juvenile rheumatoid arthritis Acta Paediatr Scand 68 423 1979 —41 children with juvenile rheumatoid arthritis (JRA) and 6 with postinfectious arthropathies aged 3–15 years were treated with acetylsalicylic acid for 14 days during which time the patients were hospitalized Three different acetylsalicylic acid preparations were used a microencapsulated form an enteric coated form and standard acetylsalicylic acid tablets Serum salicylate concentrations were measured by Trinder s photometric method With doses of 90–120 mg/kg/day symptoms of salicylism appeared in about 50% of the cases Daily doses of 2 g/m² (not exceeding 70 mg/kg) proved relatively safe in this study whereas symptoms and signs of intoxication appeared at doses exceeding 3 g/m /day In this respect there were no significant differences between the three acetyl salicylic acid preparations used The results of this study also suggest that the serum salicy late concentrations should not exceed 2 000 µmol/l (about 27 mg/100 ml) The symptoms of salicylism correlated closely with serum salicylate levels which in turn correlated well with the dosage in g/m² Elevation of serum aspartate aminotransferase was noted in 1/3 of the cases All of these had a dose exceeding 2 g/m² and the frequency of elevated enzyme activities increased with increasing dosage In the group receiving enteric-coated form of acetylsalicylic acid there were fewer positive benzidine tests (12%) than in the two other groups (22–28%)

KEY WORDS Salicylates juvenile rheumatoid arthritis aspartate aminotransferase

In order to achieve an anti inflammatory effect of acetylsalicylic acid (ASA) doses approaching toxic ones may be required (1 7) However the dose-dependent pharmacokinetics of salicylate make the dosage of ASA problematic particularly in children At high serum levels the elimination of salicylate is very slow and this will easily lead to accumulation of the drug Not all authors have accepted the necessity for the high dosage of salicylates (2 9 10 16 39) and numerous warnings have been published concerning the toxicity of ASA both in adults and in children (11 14 15 24)

In children with juvenile rheumatoid arthritis (JRA) the daily dosage of ASA recommended in modern textbooks is 90–100 mg/kg or even higher (3 4 12 34 35 36 38 40 43) When related to the body surface area the recommended daily dose is 3 0 g/m² (8)

Our preliminary results (17 27 28 29 30) which are in agreement with earlier warnings suggest that the widely accepted high dosage recommendations may be incorrect as the risk of salicylate intoxication seems to be very high Therefore we conducted a prospective study to assess the limits of safe ASA dosages for children

PATIENTS AND METHODS

47 children aged 3–15 years were divided into three groups 17 children received ASA in enteric-coated tablets (Primaspan® Lääke OY) ?1 children received it in micro-

Table 1. Age and sex of the patients in different treatment groups

Treatment	3–5 years ♀	3–5 years ♂	6–10 years ♀	6–10 years ♂	11–15 years ♀	11–15 years ♂	Total
Enteric coated ASA Primaspan®	1	–	3	7	5	1	17
Microencapsulated ASA Medisyl®	4	1	5	3	4	4	21
Aspirin® Bayer	–	–	6	–	3	–	9
Total							47

encapsulated tablets (Medisyl® Medica) and 9 children received standard uncoated ASA tablets (Aspirin® Bayer). The age and sex of the children in these three groups are shown in Table 1.

ASA was administered for 14 days, during which time the patients remained in hospital for close observation. All children entered the study with the permission of their *parents*.

The daily dosage of ASA was divided in three doses *administered at 8 a m, 2 p m and at 9 p m*.

41 patients suffered from JRA and 6 patients from post infectious arthropathies. None of them had proteinuria. Other non steroidal anti inflammatory agents were withdrawn before the trial. During the test six patients continued their maintenance gold therapy and 12 patients their therapy with hydroxychloroquine. Six patients received prednisolone during the trial, five of these intermittently (5–15 mg as single dose every other morning) and one continuously (7.6 mg every morning).

Before and during salicylate treatment the following laboratory tests were performed at weekly intervals: acid–base balance, ESR (erythrocyte sedimentation rate), Hb (haemoglobin), *WBC (white blood cell count)*, thrombocytes, S ASAT (serum aspartate aminotransferase), bleeding time and P+P prothrombin test (thromboplastin time, Simplastin A®).

The levels of *S ASAT accepted as normal (33)* were <45 U/l in patients aged 2–9 years and <35 U/l in those over 9 years of age. Bleeding time <5 min was considered as normal, as well as a value of P+P prothrombin test between 0.70–1.30.

The possible presence of occult blood in stools was checked daily with the benzidine test. The Addis count test for erythrocytes and leukocytes in urine was done twice a week. An erythrocyte excretion rate of more than 1000 erythrocytes/min and a leukocyte excretion rate of more than 2000 leukocytes/min were considered pathological.

Serum salicylate levels were determined by Trinder's photometric method (42). The precision of the method is 14.9 μmol/l (S D) at the level 1000 μmol/l ($n=109$). The blood samples were drawn every morning at 8 a m before the first daily dose of ASA. On the first, fifth and tenth day of medication additional samples were collected at noon and at 6.30 p m.

RESULTS

The mean daily dose of ASA in the whole group of 47 patients was 89±15 mg/kg (S D) (range 52.5–117 mg/kg). When calculated in *square meters of body surface area the mean daily dose was 2.4±0.5 g/m²* (range 1.4–3.7 g/m²).

The first symptoms of salicylism appeared on the fifth day of therapy. 19 out of 47 patients (40.5%) developed toxic symptoms and their treatment was therefore discontinued after a medication period varying from 5 to 13 days.

The syndrome of salicylism was mainly manifested by headache, tinnitus, dizziness, difficulty in hearing, impaired vision, sweating, nausea, vomiting, nasal congestion and slight hyperpyrexia. In five patients, all over 12 years old and weighing more than 40 kg, a more severe salicylate intoxication was characterized by hyperventilation, mental confusion, lassitude, restlessness, irritability, hyperthermia and disturbances in acid–base balance, mainly respiratory alkalosis. The treatment was discontinued on the 6th–7th day in these cases. In one girl aged 13 years hepatotoxicity and encephalopathy ("Reye's syndrome") was encountered. This began with

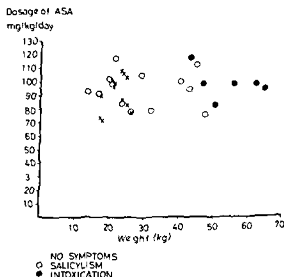


Fig. 1. Relation of the body weights of the patients and the daily dosage of ASA in mg/kg to the toxic symptoms.

Table 2 *Relation of the daily dosage of ASA (mg/kg and g/m²) to the development of symptoms of salicylism and to increased levels of S ASAT*

Daily dose of ASA	No of patients	Symptoms of salicylism No (%)	Increased S ASAT No (%)
≤70 mg/kg	7	– (0)	(?8)
71 90 mg/kg	15	5 (33)	7 (47)
91 1 0 mg/kg	?5	14 (56)	6 (4)
<? g/m²	7	– (0)	– (0)
? ? 9 g/m	35	14 (40)	12 (34)
≥3 g/m²	5	5 (100)	3 (60)

Table 3 *Salicylate serum levels related to the symptoms of salicylism*

Salicylate serum levels	No of patients	Salicylism No (%)
<1 100 μmol/l (<15 mg/100 ml)	8	– (0)
1 100–1 600 μmol/l (15–?? mg/100 ml)	8	2 (5)
1 610–2 300 μmol/l (??–3? mg/100 ml)	19	6 (31)
? 310–2 900 μmol/l (3?–40 mg/100 ml)	7	6 (86)
? 910–3 600 μmol/l (40–50 mg/100 ml)	5	5 (100)
Total	47	19

subfebrile temperature and progressed to mental confusion restlessness hyperventilation hyperpyrexia haematemesis vertigo convulsions and coma The symptoms appeared on the 10th day of treatment with a daily dose of 8? mg/kg (2 64 g/m²) This case is described in detail elsewhere (37)

Symptoms of salicylism or severe salicylate intoxication were observed in all children weighing more than 40 kg if the dosage of ASA was 80 mg/kg/day or higher (Fig 1)

In Table 2 the daily dosages of ASA are related to toxic symptoms

Serum salicylate levels related well to the symptoms of salicylism (Table 3) In each individual case the mean salicylate serum level was calculated from the morning values obtained during the period of steady state If steady state was not reached the last morning value before the discontinuation of medication was used

The daily dosage of ASA calculated in g/m² correlated well with the salicylate serum levels (Fig 2) whereas the dosage in mg/kg did not (Fig 3) Different salicylate preparations used in these tests did not show any differences in this respect According to these results the therapeutic serum salicylate levels should not exceed 2 000 μmol/l (about 27 mg/100 ml)

Acid–base balance disturbances during salicylate therapy manifested themselves mainly as depletion of the arterial P_{CO_2} and standard bicarbonate. Also patients without any clinical symptoms of salicylism showed depletion of P_{CO_2} to 2 95–3 9 kPa in 14 cases and standard bicarbonate to 20 mmol/l or less in six cases

In children displaying no clinical symptoms of salicylism (28 patients) drug administration continued for a full fortnight Steady state in salicylate serum levels was reached on the 3rd to 7th day of treatment In line with the fact that the apparent half life of salicylate elimination increases with increasing dose (18 32) the time interval needed to reach steady state in salicylate serum levels became longer the higher the fixed daily dose was With doses as high as 2 9 g/m /day steady state was not achieved until the 7th or 8th day from the start of the treatment

Fig 4 presents mean serum salicylate levels obtained during the two weeks of treatment with the three different types of ASA preparations The mean dosage in the three groups of patients was virtually identical At the beginning of the treatment the serum levels rose more slowly in the Primaspan group reflecting the slow release character of this enteric coated tablet (41) During steady state there were no significant differences between salicylate serum levels in morning samples in the different groups A tendency towards decreasing serum salicylate levels was found after 5 days which is in agreement with the findings by Muller et al (26)

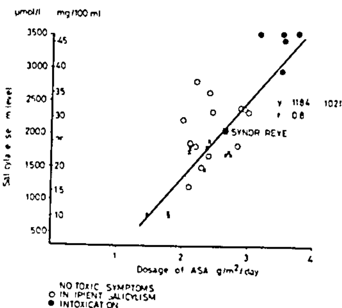


Fig 2 Correlation of salicylate serum levels with the daily dosage of ASA per m² of body surface area

Elevation of the serum aspartate amino transferase activity (S ASAT) from normal to pathological values was noted in 12 girls and 3 boys (32%) The abnormal values remained below 100 U/l except in 3 cases in whom the respective peak values were 203 252 and in the patient with toxic encephalopathy 2 535 U/l Symptoms of salicylism were observed in five of these 15 cases and apparent salicylate intoxication in two No eosinophilia or other features suggestive of drug allergy could be detected in these patients

Elevated values of S ASAT were noted during the first week of treatment in 4 cases (on the 5th or 6th day) Two of these patients had symptoms of salicylism and two displayed obvious signs of salicylate intoxication In all these cases salicylate therapy was discontinued after the first week In eleven other patients the elevated values of S ASAT were noted after 10 to 14 days from the start of the salicylate treatment

Elevated values of S ASAT returned to normal usually within one week after discontinuation of salicylate treatment Subsequently it was possible to resume ASA medication at reduced dosage without increase in enzyme activity

All but one of the patients with elevated S ASAT values had serum salicylate levels $\geq$1810 µmol/l ($\geq$25 mg/100 ml) The frequency of elevated S ASAT values increased with increasing dosage of ASA (Table 2) the lowest daily dose accompanied by pathological values of S ASAT being 2 g/m On the other hand there was no strict correlation between the occurrence of elevated S ASAT activity and the development of salicylism Nor did elevated values of S ASAT show any correlation with the clinical activity of rheumatoid arthritis or ESR

In Table 4 side effects are listed separately for each group of patients receiving a particular salicylate preparation

Benzidine tests for blood in faeces were occasionally positive in ten cases All but one changed to negative at subsequent controls despite continued salicylate treatment No decrease in haemoglobin values was observed in these patients

Positive benzidine tests were less frequent in the group receiving the enteric coated tablets of ASA than in the others Otherwise the side effects were similar in all three groups

Haematuria and leukocyturia occurred in 21–25% of the cases The number of leuko

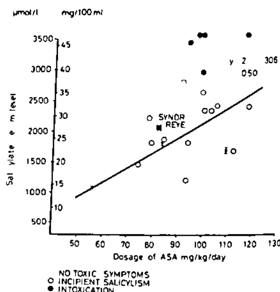


Fig 3 Correlation of salicylate serum levels with the daily dosage of ASA per kg of body weight

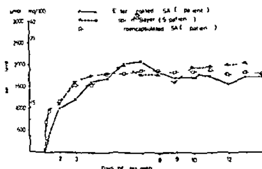


Fig 4 Mean salicylate serum levels with different ASA preparations with approx identical daily dosage x—x Enteric coated ASA (Primaspan®) 5 patients Mean daily dose 90±9 6 mg/kg (4±0 ?6 g/m²) ●—● Uncoated ASA tablets (Aspirin®) 5 patients Mean daily dose 85 2±15 3 *mg/kg* (? 5±0 ? *g/m*) □—□ *Microencapsulated* ASA (Medisyl®) 7 patients Mean daily dose 9 3± 16 mg/kg (? 56±0 ? g/m²)

cytes was rising to 10 000 cells/min or more in 5 cases and that of erythrocytes to more than 3 500 cells/min in two cases

Prolonged bleeding time or pathological values in P+P prothrombin test did not relate to clinical symptoms in this study All five patients showing pathological values in the P+P prothrombin test (the values being between 0 19–0 65) had symptoms of salicylism two of them also increased values of S ASAT

DISCUSSION

Salicylic acid is eliminated by urinary excretion of the unchanged compound (glomerular filtration with passive tubular reabsorption) and by metabolic conversion to salicyluric acid (conjugation with glycine) salicyl phenolic glucuronide salicyl acyl glucuronide and gentisic acid (44) The kinetics of salicylic acid are dose-dependent because the formation of salicyluric acid and salicyl phenolic glucuronide becomes saturated already at therapeutic dosage (18 22 23 32) According to Levy (18) the relationship between the dose and the time required to eliminate half the salicylate amount is as follows (*a*) When the dose is so small that all elimination processes follow first-order kinetics the half life is about 2 9 h (*b*) When the dose is so large that the contribution of the salicyluric acid formation process to the elimination of salicylate is negligible the half life increases to about 22 h At such high dosage elimination occurs mainly by salicylate excretion and salicylglucuronide formation (*c*) When the administered dose is of intermediate size the formation of salicyluric acid can significantly contribute to the total elimination of salicylate Considerable inter and intrasubject variations can be expected in maximum half lives of salicylate elimination even up to 41 h The results of the present study are in accordance with this concept

The renal clearance of salicylic acid is extremely sensitive to urinary pH (21 22) Administration of antacids with aspirin can cause an appreciable reduction of serum salicylate levels (20) This fact may offer at least a partial explanation of the differences between the results reported (5 6 17 30)

The long metabolic half life of salicylates at high serum levels permits more widely spaced administration of the drug per 24 h In the present study a t i d dosage reigmen was chosen instead of a four or five times daily regimen to avoid awakening of the children at midnight Our clinical experience is in accordance with recent data from Levy et al (19) that at sufficiently high daily dosage of ASA toxic symptoms cannot be avoided by dividing the total daily dose of ASA into smaller increments given at shorter intervals

According to the results of this study a daily dosage of ASA 2 g/m² (not more than 70 mg/kg) has proved to be relatively safe It is important to avoid loading the patients with heavy doses of ASA which exceed the elimination capacity of the body

An inappropriate method of calculating the dose may contribute to the appearance of salicylate toxicity For example a modern textbook recommends that in order to achieve a blood salicylate level of 20 mg/100 ml (1450 µmol/l) a dose of 3 g/m /24 h is adequate (31) This statement seems to be incorrect or at

Table 4. *Frequency of side effects in different groups*

	Enteric coated ASA Primaspan®	Microcrystall. ASA MEDISYL®	Aspirin® Bayer	Total
Number of patients	17	21	9	47
Dosage of ASA, mg/kg/day (mean ±S.D.)	87±16.8	90±15	88.5±13	89±15
Dosage of ASA, g/m²/day (mean ±S.D.)	2.4±0.5	2.5±0.6	2.4±0.2	2.4±0.5
Faecal blood loss				
No.	2	6	2	10
%	12	28	22	21
Haematuria >1000 ER/min				
No.	4	6	–	10
%	23	28		21
Leukocyturia >2000 Leuk/min				
No.	4	8	1	13
%	23	38	11	25
Elevated S-ASAT				
No.	5	7	3	15
%	29	33	33	32
Pathol. P+P prothrombin test				
No.	3	2	–	5
%	17.5	9.5		10.5
Prolonged bleeding time				
No.	3	3	1	7
%	17.5	14	11	15
Salicylism				
No.	8	4	2	14
%	47	19	22	30
Intoxication				
No.	2	3	–	5
%	12	14		10
Hepatotoxicity and encephalopathy				
No.	–	1	–	1
%		4		2

least misleading. With a constant daily dose of 3 g/m² of unbuffered ASA, the serum levels achieved during steady state are much higher, i.e. about 35 mg/100 ml (2 540 μmol/l) (Fig. 2) and are very likely to lead to intoxication.

In this study the elevations of enzyme activity of serum aspartate aminotransferase were related to high salicylate serum levels though not necessarily with symptoms of salicylate *intoxication*. The "threshold" toxic salicylate dosage in the present study appeared to be 2 g/m² of body surface area per day. Calculation of the dose *in mg per kg* of body weight is less satisfactory than per square meter of body surface. None of the patients included in the present study showed any liver function disturbances before the tests. Among the patients who reacted with an elevated S-ASAT were also 4 children with post-infectious arthropathies. Thus the possible systemic manifestation of JRA did not seem to be the primary cause of liver damage. Moreover, it is still the subject of debate whether a moderate rise in transaminases reflects hepatic toxicity or a change in enzyme turnover. The transient nature of the S-ASAT elevation during salicylate treatment suggests also that the toxic effects of salicylates are relatively benign, but *also that transaminase values should be monitored in all children receiving long-term sali*

cylate therapy (13 25) Wolfe and coworkers (45) have pointed out that the continuation of ASA therapy after the onset of hepatic dysfunction can result in more severe liver disease The case of hepatotoxicity and encephalopathy might be an example of this possibility (37)

ACKNOWLEDGEMENTS

This study was supported by Paulo Foundation and by Foundation for Paediatric Research Turku Fund

REFERENCES

1 Boardman P L & Hart F D Clinical measurement of the antiinflammatory effects of salicylates in rheumatoid arthritis *B Med J 4* .64 1967

Bywaters E G L The treatment of rheumatic fever *T ans Med Soc Lond 72* 06 19 6

3 Calabro J J Juvenile rheumatoid arthritis *In* L J Hollander & D J McCarty Jr (eds) *Arthritis and allied conditions* Lea & Febiger Philadelphia 197? 8th ed p 399

4 Calabro J J Diseases of connective tissue Juvenile rheumatoid arthritis *In* S S Gellis & B M Kagan (eds) *Current pediatric therapy 6* W B Saunders Company Philadelphia 1973 p 377

5 Calabro J J Dosage of salicylates for children with juvenile rheumatoid arthritis *In* S S Gellis (ed) *Year book of pediatrics* Year Book Medical Publishers Chicago 1977 p 384

6 Calabro J J Burnstein St L & Staley H L Dosage of salicylates for children with juvenile rheumatoid arthritis Letter to the editor *Scand J Rheumatol 5* ?51 1976

7 Calabro J J & Paulus H E Anti inflammatory effect of acetylsalicylic acid in rheumatoid arthritis *Clin O thop 71* 1 4 1970

8 Fanconi G & Wallgren A *Lehrbuch der Pad atrie* Benno Schwabe & Co Verlag Basel and Stuttgart 1956 p 94

9 Fashena G L & Walker J N Salicylate intoxication Studies on the effects of sodium salicylate on prothrombin time and alkali reserve *Am J Dis Child* 68 396 1944

10 Fischel E E The use of salicylate in rheumatic fever *Bull Rheum Dis 2* 9 195

11 Graham J D P & Parker W A The toxic manifestations of sodium salicylate therapy *Q J Med 17* 153 1948

1 on Harnack G A *A neimitteldos erung im Kin d s lte* Voraussetzungen Methoden und Ergebn se systematischer Do isbestimmungen Georg Thieme Verlag Stuttgart 1965 p 40

13 Hinkel G K Korth G & Lorenz K Salizylat bedingte toxische Hepatosen bei Kindern *Das deutsche Ges ndheitswesen 3?* 164 1977

14 Holt K S Salicylates in rheumatic fever Difficulties experienced in treating children with large doses *Lancet II* 1197 1954

15 Jager B V & Alway R The treatment of acute rheumatic fever with large doses of sodium salicylate With special reference to dose management and toxic manifestations *Am J Med Sci 211* ?73 1946

16 Keith J D & Ross A Observations on salicylate therapy in rheumatic fever *Can Med Assoc J 52* 554 1945

17 Laaksonen A L & Yrjana T Die Dosierung der Salizylatpraparate bei juveniler Rheumatoidarthritis *Kinderaer*?*l Prax 43* ?63 1975

18 Levy G Pharmacokinetics of salicylate elimination in man *J Pharm Sci 54* 959 1965

19 Levy G & Giacomini K M Rational aspirin dosage regimens *Clin Pharmacol Ther 23* 247 1978

?0 Levy G Lampman T Kamath B L & Garrettson L K Decreased serum salicylate concentrations in children with rheumatic fever treated with antacid *N Engl J Med 293* 3?3 1975

?1 Levy G & Leonards J R Urine pH and salicylate therapy *JAMA 217* 81 1971

?? Levy G & Tsuchiya T Salicylate accumulation kinetics in man *N Engl J Med 287* 430 1972

3 Levy G Tsuchiya T & Amsel L P Limited capacity for salicyl phenolic glucuronide formation and its effect on the kinetics of salicylate elimination in man *Clin Pharmacol Ther 13* ?58 197?

?4 Levy G & Yaffe S J The study of salicylate pharmacokinetics in intoxicated infants and children *Clin Toxicol 1* 409 1968

5 Miller J J III & Weissman D B Correlations between transaminase concentrations and serum salicylate concentration in juvenile rheumatoid arthritis *Arthritis Rheum 19* 115 1976

6 Muller F O Hundt H K L & Muller D G Pharmacokinetic and pharmacodynamic implications of long term administration of non steroidal anti inflammatory agents *Int J Clin Pha macol Biopharm 15* 397 1977

7 Makela A L Naproxen in the treatment of juvenile rheumatoid arthritis Metabolism safety and efficacy *Scand J Rheumatol 6* 193 1977

8 Makela A L & Yrjana T Salicylatdosering för barn med juvenil reumatoid artrit *Astra nytt 12* 5?4 1974

?9 Makela A L & Yrjana T The toxicity of high dosage salicylate therapy in children *EULAR/WHO wo kshop on the care of rheumatic children* March 21– 4/25 Oslo Norway 1977

30 Makela A L Yrjana T & Haapasaari J Dosage of salicylates for children with juvenile rheumatoid arthritis A preliminary report *Scand J Rheumatol 4* .50 1975

31 Vaughan V C & McKay R J *Nelson textbook of pediatrics* W B Saunders Company Philadelphia 1975 10th ed p 1719

3? Paulus H E Siegel M Mongan E Okun R & Calabro J J Variations of serum concentrations and half life of salicylate in patients with rheumatoid arthritis *Arthritis Rheum 14* 527 1971

Table 4. *Frequency of side effects in different groups*

	Enteric coated ASA Primaspan®	Microcrystall. ASA MEDISYL®	Aspirin® Bayer	Total
Number of patients	17	21	9	47
Dosage of ASA, mg/kg/day (mean ± S.D.)	87±16.8	90±15	88.5±13	89±15
Dosage of ASA, g/m²/day (mean ± S.D.)	2.4±0.5	2.5±0.6	2.4±0.2	2.4±0.5
Faecal blood loss				
No.	2	6	2	10
%	12	28	22	21
Haematuria >1000 ER/min				
No.	4	6	–	10
%	23	28		21
Leukocyturia >2000 Leuk/min				
No.	4	8	1	12
%	23	38	11	25
Elevated S-ASAT				
No.	5	7	3	15
%	29	33	33	32
Pathol. P+P prothrombin test				
No.	3	2	–	5
%	17.5	9.5		10.5
Prolonged bleeding time				
No.	3	3	1	7
%	17.5	14	11	15
Salicylism				
No.	8	4	2	14
%	47	19	22	30
Intoxication				
No.	2	3	–	5
%	12	14		10
Hepatotoxicity and encephalopathy				
No.	–	1	–	1
%		4		2

least misleading. With a constant daily dose of 3 g/m² of unbuffered ASA the serum levels achieved during steady state are much higher, i.e. about 35 mg/100 ml (2 540 µmol/l) (Fig. 2) and are very likely to lead to intoxication.

In this study the elevations of enzyme activity of serum aspartate aminotransferase were related to high salicylate serum levels though not necessarily with symptoms of salicylate intoxication. The threshold toxic salicylate dosage in the present study appeared to be 2 g/m² of body surface area per day. Calculation of the dose in mg per kg of body weight is less satisfactory than per square meter of body surface. None of the patients included in the present study showed any liver function disturbances before the tests. Among the patients who reacted with an elevated S-ASAT were also 4 children with post infectious arthropathies. Thus the possible systemic manifestation of JRA did not seem to be the primary cause of liver damage. Moreover it is still the subject of debate whether a moderate rise in transaminases reflects hepatic toxicity or a change in enzyme turnover. The transient nature of the S-ASAT elevation during salicylate treatment suggests also that the toxic effects of salicylates are relatively benign, but also that transaminase values should be monitored in all children receiving long term sali-

Acta Pædiatr Scand 68 431–434 1979

CORRELATION BETWEEN AGE AND PLASMA LEVEL/DOSAGE RATIO FOR PHENOBARBITAL IN INFANTS AND CHILDREN

L N ROSSI L M NINO and N PRINCIPI

From the Department of Paediatrics University School of Medicine Milan Italy

ABSTRACT Rossi L N Nino M L and Principi N (Department of Paediatrics University School of Medicine Milan Italy) Correlation between age and plasma level/ dosage ratio for phenobarbital in infants and children Acta Paediatr Scand 68 431 1979 —123 plasma concentration measurements of phenobarbital were obtained from 82 children (2 months – 6 1/2 years old) at steady state conditions The plasma level/dosage ratio has been found to have a highly significant correlation with the age of the patient both for dosage in mg/kg and in mg/m The ratio increases with the increase in the age of the patient at a rate which is greater for dosages expressed on the basis of body weight Moreover at least for body weight related dosages this increase is relatively high in the first year of life becoming less marked after Practical indications are given about the required dosage of phenobarbital in different groups of ages from 2 months up to 6 1/2 years It is recommended however to regularly measure the plasma level of the drug in infants and children treated for long periods of time

KEY WORDS Anticonvulsants developmental pharmacology infant phenobarbital pre school children

Even if an exact correlation between plasma level and the therapeutic effect of phenobarbital (PB) is not established (1 5) the therapeutic concentration range is generally considered to be 10–25 μg/ml independent of the patient s age (1 2 3 5)

A correlation was demonstrated between the plasma concentration and dosage of PB in adults One mg/kg/day gives a plasma concentration of about 10 μg/ml 2 mg/kg/day gives a concentration of about 20 μg/ml and so forth (1 9) The PB dosage necessary to reach the same plasma level is higher in children than in adults and considering the relatively few data published it seems that the younger the child the greater the PB dosage needed to reach the same plasma level (10 11) In fact the plasma level/dosage ratio was found significantly higher at 10–14 years (6 9±0 3 (S D)) than at 1–6 years (4 8±0 2) (11)

There is however no exact knowledge about the correlation between the level/dosage ratio of PB and the age of children during the first postnatal years Some of our recent observations seem to be useful in clarifying the change in the ratio from 2 months up to 6 1/2 years of age

METHODS

1 3 plasma PB concentrations were obtained from 82 children (? months – 6 1/ years old) All these patients were being treated for epilepsy or febrile convulsions and PB was administered once every 12 hours No other drugs were administered Twenty six children had two or three drug determinations during a period from 3 to 8 months after the first determination The greatest interval between the beginning of the therapy and the last drug administration was 7 years All children were free of symptoms and signs of liver or kidney failure before and during therapy so that it can be assumed that major kidney or liver illnesses did not play a role in modifying the plasma level of PB (6 8) In 31 children SGOT SGPT prothrombin activity urea creatinine blood analysis and standard urine analysis could also be performed before the beginning of the therapy and were normal Beginning after 3 weeks of therapy and with parental informed consent blood for the determination of the drug level was drawn 5–7 hours after the last administration of the drug The drug concentration was determined by gas-liquid chromatographic technique according to MacGee s meth

33 Penttila I M Jokela H A Viitala A J Heikkinen E Nummi S Pystynen P & Saastamoinen J Activities of aspartate and alanine aminotransferases and alkaline phosphatase in sera of healthy subjects *Scand J Clin Lab Invest 35* 275 1975
34 Schaller J & Wedgwood R J Diseases of connective tissue (The so called collagen diseases) Juvenile rheumatoid arthritis *In* S S Gellis & B M Kagan (eds) *Current pediatric therapy 4* W B Saunders Company Philadelphia 1970 p 536
35 Schaller J G & Wedgwood R J Rheumatic diseases Juvenile rheumatoid arthritis *In* V C Vaughan & R J McKay (eds) *Nelson textbook of pediatrics* W B Saunders Company Philadelphia 1975 p 530
36 Shanks R A Collagen or connective tissue diseases *In* J O Forfar & G C Arneil (eds) *Textbook of paediatrics* Churchill Livingstone Edinburgh 1973 p 1676
37 Sillanpaa M Makela A L & Koivikko A Acute liver failure and encephalopathy (Reye s syndrome?) during salicylate therapy *Acta Paediatr Scand 64* 877 1975
38 Sills E M Diseases of connective tissue (The so called collagen diseases) *In* S S Gellis & B M Kagan (eds) *Current pediatric therapy 7* W B Saunders Company Philadelphia 1976 p 360
39 Stevens D L & Kaplan D B Salicylate intoxication in children report of 4 cases *Am J Dis Child 70* 331 1945
40 Stollerman G H Connective tissue diseases Juvenile rheumatoid arthritis *In* H L Barnett & A H Einhorn (eds) *Pediatrics* Appleton Century-Crofts New York 1972 15 ed p 506
41 Tikkanen R & Strandman D Cellulose acetate hi phthalate CAP as enteric coating material The absorption of acetylsalicylic acid from the CAP coated enteric tablets *Farmaseuttinen Aikakauslehti Farmaseutisk Notisblad 74* 59 1965
42 Trinder P Rapid determination of salicylate in biological fluids *Biochem J 57* 301 1954
43 Wedgwood R J & Schaller J Juvenile rheumatoid arthritis (Still s disease) *In* W E Nelson V C Vaughan & R J McKay (eds) *Textbook of pediatrics* W B Saunders Company Philadelphia 1969 p 517
44 Williams R T *Detoxication mechanisms* Chapman & Hall London 1959 2nd ed p 359
45 Wolfe J D Metzger A L & Goldstein R C Aspirin hepatitis *Ann Intern Med 80* 74 1974

Submitted May 3 1978
Accepted Sept 25 1978

(A L M) Department of Paediatrics
Turku University Hospital
Kiinamyllynkatu 4–8
20520 Turku 52
Finland

Acta Pædiatr Scand 68 431–434 1979

CORRELATION BETWEEN AGE AND PLASMA LEVEL/DOSAGE RATIO FOR PHENOBARBITAL IN INFANTS AND CHILDREN

L N ROSSI L M NINO and N PRINCIPI

From the Department of Paediatrics University School of Medicine Milan Italy

ABSTRACT Rossi L N Nino M L and Principi N (Department of Paediatrics University School of Medicine Milan Italy) Correlation between age and plasma level/ dosage ratio for phenobarbital in infants and children Acta Paediatr Scand 68 431 1979 —123 plasma concentration measurements of phenobarbital were obtained from 82 children (2 months – 6 1/2 years old) at steady state conditions The plasma level/dosage ratio has been found to have a highly significant correlation with the age of the patient both for dosage in mg/kg and in mg/m The ratio increases with the increase in the age of the patient at a rate which is greater for dosages expressed on the basis of body weight Moreover at least for body weight related dosages this increase is relatively high in the first year of life becoming less marked after Practical indications are given about the required dosage of phenobarbital in different groups of ages from 2 months up to 6 1/2 years It is recommended however to regularly measure the plasma level of the drug in infants and children treated for long periods of time

KEY WORDS Anticonvulsants developmental pharmacology infant phenobarbital preschool children

Even if an exact correlation between plasma level and the therapeutic effect of phenobarbital (PB) is not established (1 5) the therapeutic concentration range is generally considered to be 10–25 μg/ml independent of the patient s age (1 2 3 5)

A correlation was demonstrated between the plasma concentration and dosage of PB in adults One mg/kg/day gives a plasma concentration of about 10 μg/ml 2 mg/kg/day gives a concentration of about 20 μg/ml and so forth (1 9) The PB dosage necessary to reach the same plasma level is higher in children than in adults and considering the relatively few data published it seems that the younger the child the greater the PB dosage needed to reach the same plasma level (10 11) In fact the plasma level/dosage ratio was found significantly higher at 10–14 years (6 9±0 3 (S D)) than at 1–6 years (4 8±0 2) (11)

There is however no exact knowledge about the correlation between the level/dosage ratio of PB and the age of children during the first postnatal years Some of our recent observations seem to be useful in clarifying the change in the ratio from 2 months up to 6 1/2 years of age

METHODS

123 plasma PB concentrations were obtained from 82 children (2 months – 6 1/2 years old) All these patients were being treated for epilepsy or febrile convulsions and PB was administered once every 12 hours No other drugs were administered Twenty six children had two or three drug determinations during a period from 3 to 8 months after the first determination The greatest interval between the beginning of the therapy and the last drug administration was 2 years All children were free of symptoms and signs of liver or kidney failure before and during therapy so that it can be assumed that major kidney or liver illnesses did not play a role in modifying the plasma level of PB (6 8) In 31 children SGOT SGPT prothrombin activity urea creatinine blood analysis and standard urine analysis could also be performed before the beginning of the therapy and were normal Beginning after 3 weeks of therapy and with parental informed consent blood for the determination of the drug level was drawn 5–7 hours after the last administration of the drug The drug concentration was determined by gas-liquid chromatographic technique according to MacGee s meth

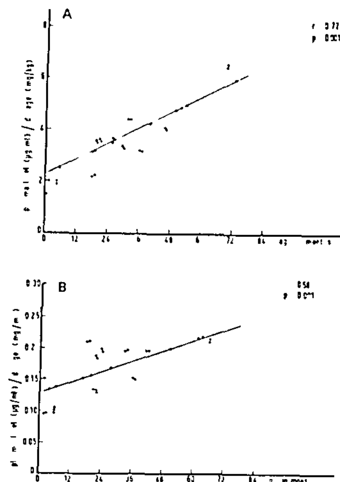


Fig. 1. Correlation between age and plasma level/dosage ratio for PB in children. *A*: dosage in mg/kg. *B*: dosage in mg/m²

od (7). A Fractovap Gas Chromatography Apparatus was employed equipped with a flame ionization detector (Carlo Erba). Exobarbital was used as an internal standard. PB was extracted with the following modified procedure: 1 ml of plasma acidified with 0.1 ml 1N HCl was extracted with 8 ml chloroform by gentle shaking for 30 min. 7 ml of clear chloroform layer added to 20 µg of internal standard (exobarbital 200 µg/ml ethanol solution) were dried under nitrogen stream in a water bath at 50 C. The residue was dissolved in 50 µl of tetramethyl ammonium and 1-2 µl were injected into the gas chromatograph. A calibration curve (5-30 µg/ml in fresh plasma) was carried out each time with unknown samples.

Table 1. *Plasma level/dosage ratios of PB for three different age groups*

The dosage of PB is calculated in mg/kg

2 months-1 year	1-3 years	3-6½ years
2.31±0.74 (S.D.) (S.E. 95%=0.31)	3.54±0.99 (S.D.) (S.E. 95%=0.24)	4.79±1.31 (S.D.) (S.E. 95%=0.40)

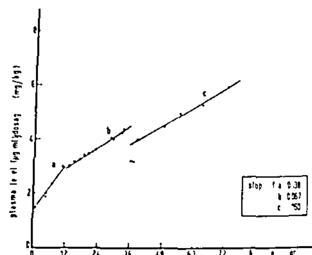


Fig. 2. Correlations between age and plasma level/dosage ratios for PB for weight related dosages in the 3 groups of children divided according to their age.

The correlations of the plasma level/dosage ratio with the age of the patient were calculated both for weight and surface area related dosages. The ratios for weight related dosages within the three groups of children divided according to age (2 months-1 year, 1-3 years, 3-6 1/2 years) were also calculated, as were their correlations with the age of the children in each group. The dosage of PB in mg/kg/day required at the different ages in order to have plasma level of 10-25 µg/ml respectively was calculated from these ratios.

RESULTS

Fig. 1 shows the correlations of the plasma level/dosage ratio with the age of the patient both for weight and surface area related dosages. The two correlations are highly significant (<0.001). The plasma level/dosage ratio increases as the age of the patient increases, this increase being greater for dosage in mg/kg than for dosage in mg/m². Table 1 shows the plasma level/dosage ratios for weight related dosages in the 3 groups of children divided according to age (2 months-1 year, 1-3 years, 3-6 1/2 years). These ratios maintain a significant correlation with the age of the patient ($p<0.01$ for the 3 lines, this significance being lower than that of the entire group as each individual group has fewer data). Fig. 2 shows the 3 lines with their respective slopes: it is clear that the rate of increase of plasma level/dosage ratio shown to

Table 2 *Dosages of PB in mg/kg/day required at the different ages in order to have plasma levels of 10–25 μg/ml respectively*

months–1 year	1–3 years	3–6 1/2 years
4–11 mg/kg	3–7 mg/kg	?–5 mg/kg

exist with the increase in age diminishes after the first year

The plasma level/dosage ratios for surface area related dosages have no significant correlation with the ages of the children in the 3 groups because the data were too dispersed

DISCUSSION

Our data prove that in steady state conditions and in the absence of liver and kidney illnesses from 2 months up to 6 1/2 years of age the plasma level/dosage ratio for PB has a statistically significant increase with the increase of the patient s age both for dosage in mg/kg and in mg/m² Moreover at least for body weight related dosages this increase is relatively high in the first year of life becoming less marked thereafter

From a practical point of view the dosages in mg/kg/day required to have PB level of 10–25 μg/ml in the 3 groups of children divided according to their age (from 2 months up to 6 1/2 years) are shown in Table 2

The increase of plasma level/dosage ratio for PB as the patient gets older presumably depends on the variations of drug metabolism during development The apparent plasma half life of the drug increases with the increase in age (4 9 10 11) and this seems to be the most important factor The role of volume of distribution variation is still undetermined as it was never thoroughly evaluated in the age period we have considered

As most of our subjects are out patients an important question is that of compliance of parents To overcome this problem children should have been hospitalized for at least 3 weeks before each drug determination This was an impossible procedure for practical and ethical reasons Therefore the variability of our data may be in part due to the irregular administration of the drug in some children On the other hand our data are so significant that it seems highly improbable to us that a lack of compliance plays an important role Considering the variability among individuals and the speed with which the level/dosage ratio for PB varies during growth it is recommended to regularly measure the plasma level of the drug in infants and children treated for long periods of time Only in this way can an adequate dosage be maintained

ACKNOWLEDGEMENT

This work has been in part supported by CNR Grant 76 00631 83

REFERENCES

1 Buchthal F & Lennox Buchthal M A Phenobarbital Relation of serum concentration to control of seizures *In* D M Woodbury J K Penry & R P Schmidt (eds) *Antiepileptic drugs* Raven Press New York 197? p 335

2 Buchthal F Svensmark P & Simonsen H Relation of EEG and seizures to phenobarbital in serum *Arch Neurol 19* 567 1968

3 Faero O Kastrup K W Melchior J C & Thorn J Phenobarbital as prophylaxis for febrile convulsions *Epilepsia 1?* 109 1971

4 Garrettson L K & Dayton P G Disappearance of phenobarbital and diphenylhydantoin from serum of children *Clin Pharmacol Ther 2* 674 1970

5 Jalling B Plasma concentrations of phenobarbital in the treatment of seizures in newborns *Acta Paediatr Scand 64* 514 1975

6 Kutt H Winters W Scherman R & McDowell F Diphenylhydantoin and phenobarbital toxicity *Arch Neurol 11* 649 1964

7 MacGee J Rapid determination of diphenylhydantoin in blood plasma by gas liquid chromatography *Anal Chem 42* 4 1 1970

8 Maynert E W Phenobarbital mephobarbital and methabarbital Absorption distribution and excretion *In* D M Woodbury J K Penry & R P Schmidt (eds) *Antiepileptic drugs* Raven Press New York 197? p 303

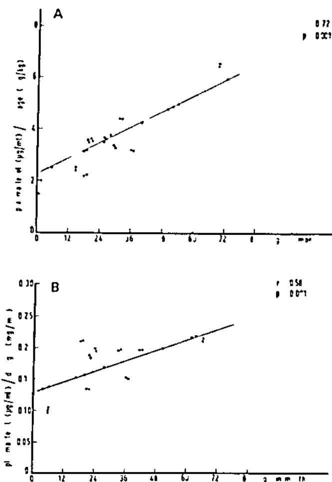


Fig 1 Correlation between age and plasma level/dosage ratio for PB in children *A* dosage in mg/kg *B* dosage in mg/m²

od (7) A Fractovap Gas Chromatography Apparatus was employed equipped with a flame ionization detector (Carlo Erba) Exobarbital was used as an internal standard PB was extracted with the following modified procedure 1 ml of plasma acidified with 0 1 ml 1N HCl was extracted with 8 ml chloroform by gentle shaking for 30 min 7 ml of clear chloroform layer added to 20 µg of internal standard (exobarbital 200 µg/ml ethanol solution) were dried under nitrogen stream in a water bath at 50 C The residue was dissolved in 50 µl of tetramethyl ammonium and 1–2 µl were injected into the gas chromatograph A calibration curve (5–30 µg/ml in fresh plasma) was carried out each time with unknown samples

Table 1 *Plasma level/dosage ratios of PB for three different age groups*

The dosage of PB is calculated in mg/kg

2 months – 1 year	1–3 years	3–6½ years
2 31±0 74 (S D) (S E 95%=0 31)	3 54±0 99 (S D) (S E 95%=0 24)	4 79±1 31 (S D) (S E 95%=0 40)

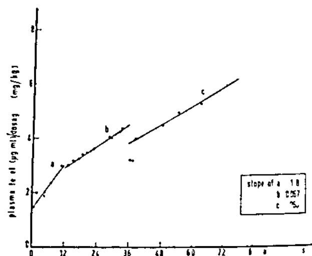


Fig 2 Correlations between age and plasma level/dosage ratios for PB for weight related dosages in the 3 groups of children divided according to their age

The correlations of the plasma level/dosage ratio with the age of the patient were calculated both for weight and surface area related dosages The ratios for weight related dosages within the three groups of children divided according to age (2 months–1 year 1–3 years 3–6 1/2 years) were also calculated as were their correlations with the age of the children in each group The dosage of PB in mg/kg/day required at the different ages in order to have plasma level of 10–25 µg/ml respectively was calculated from these ratios

RESULTS

Fig 1 shows the correlations of the plasma level/dosage ratio with the age of the patient both for weight and surface area related dosages The two correlations are highly significant (<0 001) The plasma level/dosage ratio increases as the age of the patient increases this increase being greater for dosage in mg/kg than for dosage in mg/m Table 1 shows the plasma level/dosage ratios for weight related dosages in the 3 groups of children divided according to age (2 months–1 year 1–3 years 3–6 1/2 years) These ratios maintain a significant correlation with the age of the patient ($p<0\ 01$ for the 3 lines this significance being lower than that of the entire group as each individual group has fewer data) Fig 2 shows the 3 lines with their respective slopes it is clear that the rate of increase of plasma level/dosage ratio shown to

Table 2 *Dosages of PB in mg/kg/day required at the different ages in order to have plasma levels of 10–25 μg/ml respectively*

2 months–1 year	1–3 years	3–6½ years
4–11 mg/kg	3–7 mg/kg	2–5 mg/kg

exist with the increase in age diminishes after the first year

The plasma level/dosage ratios for surface area related dosages have no significant correlation with the ages of the children in the 3 groups because the data were too dispersed

DISCUSSION

Our data prove that in steady state conditions and in the absence of liver and kidney illnesses from 2 months up to 6 1/2 years of age the plasma level/dosage ratio for PB has a statistically significant increase with the increase of the patient s age both for dosage in mg/kg and in mg/m² Moreover at least for body weight related dosages this increase is relatively high in the first year of life becoming less marked thereafter

From a practical point of view the dosages in mg/kg/day required to have PB level of 10–25 μg/ml in the 3 groups of children divided according to their age (from 2 months up to 6 1/2 years) are shown in Table 2

The increase of plasma level/dosage ratio for PB as the patient gets older presumably depends on the variations of drug metabolism during development The apparent plasma half life of the drug increases with the increase in age (4 9 10 11) and this seems to be the most important factor The role of volume of distribution variation is still undetermined as it was never thoroughly evaluated in the age period we have considered

As most of our subjects are out patients an important question is that of compliance of parents To overcome this problem children should have been hospitalized for at least 3 weeks before each drug determination This was an impossible procedure for practical and ethical reasons Therefore the variability of our data may be in part due to the irregular administration of the drug in some children On the other hand our data are so significant that it seems highly improbable to us that a lack of compliance plays an important role Considering the variability among individuals and the speed with which the level/dosage ratio for PB varies during growth it is recommended to regularly measure the plasma level of the drug in infants and children treated for long periods of time Only in this way can an adequate dosage be maintained

ACKNOWLEDGEMENT

This work has been in part supported by CNR Grant 76 00631 83

REFERENCES

1 Buchthal F & Lennox Buchthal M A Phenobarbital Relation of serum concentration to control of seizures *In* D M Woodbury J K Penry & R P Schmidt (eds) *Antiepileptic drugs* Raven Press New York 1972 p 335

2 Buchthal F Svensmark P & Simonsen H Relation of EEG and seizures to phenobarbital in serum *Arch Neurol 19* 567 1968

3 Faero O Kastrup K W Melchior J C & Thorn J Phenobarbital as prophylaxis for febrile convulsions *Epilepsia 12* 109 1971

4 Garrettson L K & Dayton P G Disappearance of phenobarbital and diphenylhydantoin from serum of children *Clin Pharmacol Ther 2* 674 1970

5 Jalling B Plasma concentrations of phenobarbital in the treatment of seizures in newborns *Acta Paediatr Scand 64* 514 1975

6 Kutt H Winters W Scherman R & McDowell F Diphenylhydantoin and phenobarbital toxicity *Arch Neurol 11* 649 1964

7 MacGee J Rapid determination of diphenylhydantoin in blood plasma by gas liquid chromatography *Anal Chem 42* 421 1970

8 Maynert E W Phenobarbital mephobarbital and methabarbital Absorption distribution and excretion *In* D M Woodbury J K Penry & R P Schmidt (eds) *Antiepileptic drugs* Raven Press New York 197 p 303

9 Svensmark O & Buchthal F Accumulation of phenobarbital in man *Epilepsia 4* 199 1963
10 Svensmark O & Buchthal F Dosage of phenytoin and phenobarbital in children *Dan Med Bull 10* 234 1963
11 Svensmark O & Buchthal F Diphenylhydantoin and phenobarbital Serum levels in children *Am J Dis Child 108* 82 1964

Submitted Sept 18 1978
Accepted Nov 17 1978

(L N R) Clinica Pediatrica
Via Commenda 9
20122 Milano
Italy

ta Pædiatr Scand 68 435–439 1979

SERUM CONCENTRATIONS OF THEOPHYLLINE IN CHILDREN FOLLOWING THE ADMINISTRATION OF DOSES GENERALLY RECOMMENDED NEW DOSAGE REGIMEN REQUIRED

K SELVIG T LINGAAS HOLMEN K AAS [2] H E RUGSTAD and K S BJERVE

From the [1]Institute of Clinical Biochemistry the [2]Allergy Institute Voksentoppen Department of Pædiatrics and the [3]Department of Surgical Research Section of Clinical Pharmacology Rikshospitalet Oslo Norway

ABSTRACT Selvig K Lingaas Holmen T Aas K Rugstad H E and Bjerve K S (Institute of Clinical Biochemistry the Allergy Institute Voksentoppen Department of Paediatrics and Department of Surgical Research Section of Clinical Pharmacology Rikshospitalet National Hospital of Norway Oslo Norway) Serum concentrations of theophylline in children following the administration of doses generally recommended new dosage regimen required Acta Paediatr Scand 68 435 1979 —Serum concentrations of theophylline following intravenous and oral administration of aminophylline were studied in asthmatic children 2–17 years of age The biological half life ($t_{\frac{1}{2}}\beta$) of theophylline varied between 165 and 495 min The results revealed that an intravenous loading dose of 6 mg of aminophylline per kg body weight was necessary in order to obtain therapeutic concentrations in children who had not received the drug for the last 6 to 8 hours The maintenance dose should be determined and controlled by use of serum concentration determinations In a group of children receiving 5 mg of aminophylline per kg body weight 3 times a day orally none had concentrations within the therapeutic range in the morning and only 39% reached therapeutic levels 2 h after the morning dose No correlation was found between the serum concentration of theophylline and the amount of drug given per kg body weight The results show that theophylline concentration analysis is necessary to obtain adequate therapeutic levels in children without risking toxic effects

KEY WORDS Theophylline children asthma serum drug concentration

Theophylline is known to be an effective bronchodilator and is widely used in the treatment of bronchospasm in asthma and chronic obstructive lung disease The drug is often administrated as aminophylline the ethylene diamine salt of theophylline

The effect of theophylline on pulmonary function is related to the serum concentration and 55 to 110 μmol/l has been accepted as the therapeutic range (6 9 13) The therapeutic index of theophylline is narrow and the frequency of gastrointestinal cerebral nervous and cardiovascular side effects is high at concentrations above the therapeutic range Serious and occasionally fatal effects such as convulsions atrial and ventricular arrhythmias and hypo- or hypertension have been reported at concentrations above 135 μmol/l (4 16)

Theophylline is biotransformed in the liver and the more water soluble metabolites are excreted in the urine (1 5) Only 10% of the drug is excreted unchanged in the urine There is a large interindividual variation in the rate of theophylline elimination (6 10) which is mainly due to different rates of metabolism In children the mean biologic half life is shorter than in adults (2 15) and therefore they usually need shorter dose intervals on higher doses than adults to maintain therapeutic serum concentrations

We have studied the drug concentrations in serum obtained after administration of conventional doses of theophylline to children

9 Svensmark O & Buchthal F Accumulation of phenobarbital in man *Epilepsia 4* 199 1963
10 Svensmark O & Buchthal F Dosage of phenytoin and phenobarbital in children *Dan Med Bull 10* 234 1963
11 Svensmark O & Buchthal F Diphenylhydantoin and phenobarbital Serum levels in children *Am J Dis Child 108* 82 1964

Submitted Sept 18 1978
Accepted Nov 17 1978

(F N R) Clinica Pediatrica
Via Commenda 9
20122 Milano
Italy

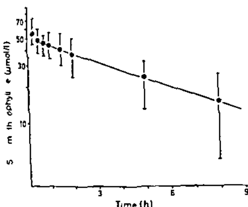


Fıg 1 Serum theophyllıne concentration as a function of time after intravenous injection of 5 mg of amınophyl line per kg body weight Mean and range from 16 chil dren are given

lıne per kg body weight ranged from 45 to 73 µmol/l and 4 to 23 µmol/l respectively The apparent volume of distribution varied from 0 37 to 0 53 l/kg while the biologic half life (β phase) varied from 165 to 495 mın (simı lar to previously reported results (2 15)) Table 2 shows that the individual biologic half life (β phase) of theophyllıne in children was independent of dose over the range from 3 mg to 5 mg of amınophyllıne per kg body weight ı e there was no discernable dose dependent kinetics in this dose range and the increase in serum concentration was propor tional to the increase in drug dose The mean biologic half life of theophyllıne in children receiving no other drugs was not statistically different from the half life found in children receiving simultaneous administration of therapeutic doses of salbutamol adrenalıne corticosteroids or disodium cromoglycate (291 min and 294 mın respectively)

The results following oral treatment with aminophyllıne are shown in Fıg 2 The mean daily dose of amınophyllıne was 16 3 mg/kg body weight ranging from 10 3 to 23 2 mg/kg This corresponds to 12 8 (8 1–18 3) mg of theophyllıne per kg body weight No correla tion could be found between such total daily doses of theophyllıne and the corresponding serum concentrations neither before the morning dose nor at the expected peak con centration 2 hours later The serum concen tration was also measured 4 and 8 hours after the morning dose and again no correlation with the daily dose was found (results not shown)

The serum concentration of theophyllıne fluctuated considerably during the day and this was strongly influenced by the dose in tervals used The children who had the drug administered 3 times during a period of 11 hours had very low serum concentrations in the morning and accumulated the drug during the day reaching a maximum serum concen tration after the evening dose in contrast to children receiving the drug every 8 hours who showed a less pronounced fluctuation of the serum concentration However although those who received the last dose at 1 a m

Table 2 *Changes in the serum concentration and biologic half life after increase in dose of theophyllıne*

4 children received an intra enous do e of 3 mg and after an interval of at least 4 days 5 mg of aminophyllıne per kg body weight (corresponding to 4 mg and 3 9 mg theophyllıne respectively) a dose increase of 63 %

Pat ent no	Theophyllıne concentration (% increase)			Biologic half life min (β phase)	
	15 min	90 min	5 h	3 mg/kg	5 mg/kg
\	59	56	79	31	336
	36	43	67	180	00
1	70	65	57	64	58
4	9	70	75	6	86

Table 1. *Serum concentrations of theophylline obtained in children 15 min after start of a single intravenous dose of aminophylline*

Dose of aminophylline (mg/kg body weight)	No. of children	Theophylline concentration after 15 min (μmol/l)[b]	Time within therapeutic area (min)[c]
3 (2.4)[a]	4	33 (33–34)	<15
5 (3.9)	16	55 (37–73)	20 (<15–75)
6 (4.7)	5	61 (47–72)	68 (<15–120)

[a] Dose recalculated as theophylline.
[b] Mean and range are given.
[c] Time within therapeutic area is calculated from semilogarithmic plots of serum concentrations versus time. Median and range are given.

SUBJECTS AND METHODS

This study includes 68 children, age 2–17 years, all hospitalized with a diagnosis of bronchial asthma. Both children and parents had consented to the study.

Intravenous administration. Twenty-one children, age 8–15 years, without any other known illness, were included in this study. They did not receive xanthine-containing drugs for 72 h prior to the study. Eleven of the children received other bronchodilators, corticosteroids or cromoglycate, but these drugs were discontinued 6 h before the study. Aminophylline[1] 3, 5 or 6 mg per kg body weight was injected over a period of 10 min. Four of the children received both 3 mg and, after an interval of at least 4 days, 5 mg per kg body weight. A Venflon® was installed in the opposite arm, and 4 ml blood were sampled before and then 15, 30, 45, 60, 90, 2 h, 5 h, 8 h and in some cases 12 h and 24 h after the start of the injection. The lung function measured as FEV₁ and the pulse rate were registered during the test period.

Oral administration. Serum concentrations of theophylline were studied in a non-selected group of 47 children admitted to the hospital and treated with aminophylline.[2] The mean daily dose was 16.3 mg/kg body weight (range 10.3–23.2). The drug was administered 2 or 3 times a day for at least 72 h before the test. Blood samples were taken immediately before and 2 h after the morning dose. Some of the children used bronchodilators, corticosteroids or cromoglycate. No change was made in this therapy during the study.

Analytical method. The serum concentration of theophylline was determined by cation exchange HPLC as previously described (12).

RESULTS

The serum concentrations of theophylline after intravenous injection of 3, 5 or 6 mg of aminophylline per kg body weight are shown in Table 1. Because of the very slow intravenous injection of the drug, it is assumed that the serum concentration 15 min after start of the injection gives a good estimate of the maximal concentration reached in each child. To obtain therapeutic concentrations of theophylline, it was necessary to give at least 5 mg of aminophylline per kg body weight as a single loading dose. At this dose 62% of the children reached assumed therapeutic values 15 min after the start of the injection. Only in 1 patient did the concentration remain within the therapeutic range for more than 1 hour. In all children, including 5 receiving 6 mg/kg body weight, the maximal serum concentration obtained was in the lower half of the therapeutic range.

Table 1 illustrates the large variations in serum concentrations of theophylline following administration at three dose levels. This is further shown in Fig. 1 in which a semilogarithmic plot of the mean serum concentration after a loading dose of 5 mg of aminophylline per kg body weight in 16 children is given. After a rapid α phase (mean $t_{\frac{1}{2}}$ 10 min), the elimination curve was rectilinear but the slope of the β phase varied, as demonstrated by the increasing range of the serum theophylline values with time. Thus the theophylline concentration 15 min and 8 hours after a single injection of 5 mg of aminophyl-

[1] Aminophylline AFI (30 mg/ml).
[2] Theodrox 3M Riker (aminophyllin 195 mg/tbl).

act this by means of therapeutic serum con centrations also in the morning aminophylline must be administrated 3 or 4 times during 24 hours However in most children it is not pos sible to give any medication during the night which necessarily results in subtherapeutic concentrations in the morning This problem could hopefully be overcome using a sus tained release preparation

ACKNOWLEDGEMENT

We thank the staff of the Clinical Department of Voksen toppen and the Department of Clinical Chemistry Riks hospitalet—and the patients!

REFERENCES

1 Cornish H H & Christman A A A study of the metabolism of theobromine theophylline and caf feine in man *J Biol Chem* ?78 315 1957

Ellis E F Koysooko R & Levy G Pharmaco kinetics of theophylline in children with asthma *Pediatrics 58* 54? 1976

3 Fixley M Shen D D & Azarnoff D L Theo phylline bioavailability A comparison of the oral absorption of a theophylline elixir and two combina tion theophylline tablets to intravenous aminophyl line *Am Rev Resp Dis 115* 955 1977

4 Jacobs M H Senior R M & Kessler G Clini cal experience with theophylline Relationship be tween dosage serum concentration and toxi ity *JAMA 235* 1983 1976

5 Jenne J W Nagasawa H T & Thompson R D Relationship of urinary metabolites of theophylline to serum theophylline levels *Clin Pl armacol Ther 19* 375 19 6

6 Jenne J W Wyse E Rood F S & MacDonald F M Pharmacokineti s of theophylline Application to adjustment of the clinical do e of aminophylline *Clin Pharmacol Ther 13* 349 197

7 Kern J W & Lipman A G Rational theophyl line therapy *Drug Intell Clin Pharm 11* 144 1977

8 Kordash T R Van Dellen R G & McCall J T Theophylline concentrations in asthmatic patients after administration of aminophylline *JAMA* ?3? 139 1977

9 Mitenko P A & Ogilvie R I Rational intravenous do es of theophylline *N Engl J Med* ?89 600 1973

10 Mitenko P A & Ogilvie R I Pharmacokinetics of intravenous theophylline *Clin Pharmacol Ther 14* 509 1973

11 Piafsky K M & Ogilvie R I Dosage of theophyl line in bronchial asthma *N Engl J Med* 29? 1?18 1975

12 Selvig K & Bjerve K S Determination of serum proxiphylline by high pressure liquid chromatogra phy *Scand J Lab Invest 37* 373 1977

13 Weinberger M M & Bronsky E A Evaluation of oral bronchodilator therapy in asthmatic children *J Pediatr 84* 421 1974

14 Welling P G Lyons L L Craig W A & Trochta G A Influence of diet and fluid on bioavailability of theophylline *Clin Pharmacol Ther 17* 475 1975

15 Zaske D E Miller K W Strem E L Austrian S & Johnson P B Oral aminophylline therapy Increased dosage requirements in children *JAMA* ?37 1453 1977

16 Zwillich C W Sutton F D Neff T A Cohn W M Matthay R A & Weinberger M M Theo-phylline induced seizures in adults Correlation with serum concentrations *Ann Intern Med 8?* 784 1975

Submitted Aug ?9 1978
Accepted Nov 17 1978

(K S) Institute of Clinical Biochemistry
Riksho pitalet
O lo 1
Norway

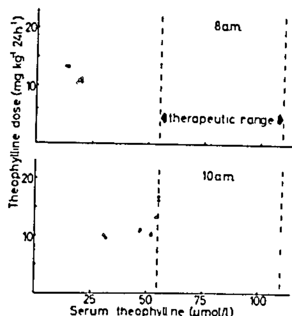


Fig. 2. Relation between the daily dose of theophylline and the serum concentration in 47 children after oral administration. The drug was administrated 2 or 3 times daily with the last dose at 7 p.m. (O) or 3 times daily with the last dose at 1 a.m. (●). Serum concentrations were determined immediately before the morning dose (8 a.m.) and 2 h afterwards (10 a.m.).

tended to have a higher concentration, all children had subtherapeutic serum concentrations in the morning as shown in Fig. 2.

None of our patients had serum theophylline concentrations above the therapeutic range and no side effects were registered in the ward. One child complained of gastrointestinal pain after he had been discharged from the hospital. On control he had a serum concentration of 47 µmol/l.

DISCUSSION

The recommended intravenous dose of aminophylline in the treatment of bronchial asthma has been 3 mg/kg body weight, corresponding to 2.4 mg of theophylline. This dose has mainly been selected from clinical data excluding the higher doses following reports of side effects. Our results show that the recommended dose does not produce therapeutic serum concentrations. In children who have not previously received theophylline therapy a loading dose of 6 mg of aminophylline per kg body weight seems to be more adequate confirming results reported by other groups (9, 11). In patients already on oral or rectal aminophylline treatment, the loading dose should usually be somewhat lower and in some children even omitted to avoid toxic serum concentrations.

Maintenance therapy can be given either as repeated intravenous injections or as continuous intravenous infusions. The infusion dose proposed to provide therapeutic serum theophylline concentrations is 0.9 mg/kg/h in adults (9). However, this dose gives theophylline concentrations above the upper therapeutic limit in more than 30% of the patients (8). Children tend to require somewhat higher doses because of a higher total clearance of the drug (2). However, because of the pronounced interindividual differences in the kinetics of the drug (Fig. 1), it is necessary to adjust the individual dose based on serum concentration determinations.

The same interindividual differences are found after oral administration of aminophylline. In this study no correlation was found between the total daily dose and the serum concentration, but some of this discrepancy may also be due to different rates of absorption of the drug (3). In other studies on the bioavailability of theophylline the peak serum concentration was reached 1 to 2 hours after oral administration (3, 7). This can, however, be strongly influenced by the diet and intake of fluid (14). In our group of children 30% were still in the absorption phase 2 hours after oral administration of the drug, as indicated by a higher concentration 4 hours after administration.

The routinely used dose of aminophylline has been 5 mg/kg body weight 3 times daily. On this dose none of the children we studied had a serum concentration within the therapeutic area in the morning and only 39% had reached therapeutic levels 2 hours after the morning dose. Bronchial obstruction on awakening in the morning is a serious problem in children with chronic asthma. To counter

Acta Pædiatr Scand 68 441–442 1979

SHORT COMMUNICATION

SALT CONTENT IN HUMAN BREAST MILK DURING THE THREE FIRST WEEKS AFTER DELIVERY

During a study of electrolyte balance in new born infants the concentration of sodium and potassium in breast milk was determined on different days after delivery

Material and Methods Mothers who were delivered at term were studied on the first ($n=5$) second ($n=5$) third ($n=17$) fourth ($n=14$) fifth ($n=19$) sixth ($n=6$) seventh ($n=5$) eighth and ninth ($n=5$) tenth and eleventh ($n=5$) and twenty second to twenty sixth ($n=12$) days after delivery In addition 19 samples were obtained 1–11 days after de livery from mothers who were delivered during the 32nd–34th gestational week A 1 ml sample of breast milk was obtained at the beginning and/or at the end of the feeding The con centration of sodium and potassium in 1/50 diluted breast milk was determined with a flame photometer

Results and Comments In 11 mothers de livered at term and studied 3–6 days after de livery the concentration of sodium and potassium in breast milk was determined both be fore and after feeding Paired t tests showed no significant differences between the samples (Na $p>0.9$ K $0.5>p>0.4$) The concentration of sodium in breast milk (Colostrum) of mothers who were delivered at term was high 61 mmol/l as expected on the first day of life (6) It then fell to about 20 mmol/l at the sec ond or third day of life and remained at this level until at least the 11th day after delivery (Fig 1) The average sodium concentration in breast milk from the 10th to the 11th day after delivery 24 6 mmol/l was significantly higher than the average sodium concentration in breast milk 3–4 days after delivery 5 1 mmol/l ($p<0.001$) The sodium concentration in breast milk 3–4 weeks after delivery was in the same range as that reported previously (3 6) The sodium concentration of breast milk in mothers delivered before term was also comparatively higher during the first weeks after delivery but was somewhat lower than that of breast milk from mothers who were delivered at term (Fig 2) The potassium con centration of breast milk is fairly constant after delivery The potassium sodium ratio in breast milk is lower during the first 10 days after delivery than later during lactation (Fig 3)

The results also suggest that infants who are not breast fed should be given salt supple ments This is probably more important for preterm infants since they have greater urinary salt losses than the full term infants (1 2 8)

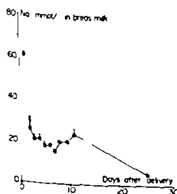


Fig 1 Sodium content in breast milk from mothers who are delivered at term The dots represent mean values and the bar ± S E

Supported by grants from the Swedish Medical Research Council (B78-19X-03644-06) and from the Research Funds of the Karolinska Institute

Acta Pædiatr Scand 68 441–442 1979

SHORT COMMUNICATION

SALT CONTENT IN HUMAN BREAST MILK DURING THE THREE FIRST WEEKS AFTER DELIVERY

During a study of electrolyte balance in new born infants the concentration of sodium and potassium in breast milk was determined on different days after delivery

Material and Methods Mothers who were delivered at term were studied on the first ($n=5$) second ($n=5$) third ($n=17$) fourth ($n=14$) fifth ($n=19$) sixth ($n=6$) seventh ($n=5$) eighth and ninth ($n=5$) tenth and eleventh ($n=5$) and twenty second to twenty sixth ($n=12$) days after delivery In addition 19 samples were obtained 1–11 days after de livery from mothers who were delivered during the 32nd–34th gestational week A 1 ml sample of breast milk was obtained at the beginning and/or at the end of the feeding The con centration of sodium and potassium in 1/50 diluted breast milk was determined with a flame photometer

Results and Comments In 11 mothers de livered at term and studied 3–6 days after de livery the concentration of sodium and potas sium in breast milk was determined both be fore and after feeding Paired *t* tests showed no significant differences between the samples (Na $p>0\ 9$ K $0\ 5>p>0\ 4$) The concentration of sodium in breast milk (Colostrum) of mothers who were delivered at term was high 61 mmol/l as expected on the first day of life (6) It then fell to about 20 mmol/l at the sec ond or third day of life and remained at this level until at least the 11th day after delivery (Fig 1) The average sodium concentration in breast milk from the 10th to the 11th day after delivery 24 6 mmol/l was significantly higher than the average sodium concentration in breast milk 3–4 days after delivery 5 1 mmol/l ($p<0\ 001$) The sodium concentration in breast milk 3–4 weeks after delivery was in the same range as that reported previously (3 6) The sodium concentration of breast milk in mothers delivered before term was also comparatively higher during the first weeks after delivery but was somewhat lower than that of breast milk from mothers who were delivered at term (Fig 2) The potassium con centration of breast milk is fairly constant after delivery The potassium sodium ratio in breast milk is lower during the first 10 days after delivery than later during lactation (Fig 3)

The results also suggest that infants who are not breast fed should be given salt supple ments This is probably more important for preterm infants since they have greater urinary salt losses than the full term infants (1 2 8)

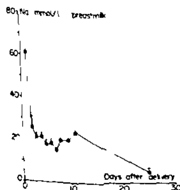


Fig 1 Sodium content in breast milk from mothers who are delivered at term The dots represent mean values and the bar 1 S E

Supported by grants from the Swedish Medical Research Council (B78-19X-03644-06) and from the Research Funds of the Karolinska Institute

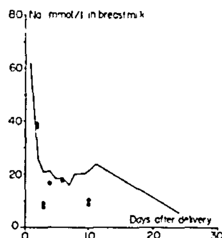


Fig. 2. Sodium content in breast milk from mothers that are delivered during the 32nd–34th gestational week (o). The line represents the average values observed in breast milk from mothers that are delivered at term.

and easily develop hyponatremia (5, 7). A sodium supplement has been reported to induce accelerated growth in preterm infants (4). The findings reported indicate that an increased active reabsorption of sodium in the mammary gland is responsible for the fall in sodium concentration of breast milk after the 10th day of delivery. The parallel changes in the potassium sodium ratio indicate that this is accomplished by an increased sodium potassium exchange.

Anita Aperia, O. Broberger, P. Herin, R. Zetterström

Department of Paediatrics
St. Göran's Children's Hospital
Box 12500
S-11281 Stockholm
Sweden

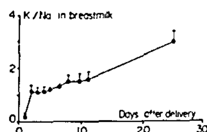


Fig. 3. Potassium sodium ratio in breast milk from mothers that are delivered at term. The dots represent the mean value and the bar 1 S.E.

REFERENCES

1. Aperia A, Broberger O, Herin P & Zetterström R. Salt balance and aldosterone excretion during the first two weeks of life in preterm and full term infants. *Acta Paediatr Scand*, submitted for publication.
2. Arant B S Jr. Developmental patterns of renal functional maturation compared in the human neonate. *J Pediatr* 92: 705, 1978.
3. Babson G. Feeding the low birth weight infant. *J Pediatr* 79: 694, 1971.
4. Chance G W, Radde I C, Willis D M & Park E. Postnatal growth of infants of <1.3 kg birth weight. Effects of metabolic acidosis, of caloric intake and of calcium, sodium and phosphate supplementation. *J Pediatr* 91: 787, 1977.
5. Day G M, Radde I C, Balfe J W & Chance G W. Electrolyte abnormalities in very low birth weight infants. *Pediatr Res* 10: 522, 1976.
6. Renner E. *Milch und Milchprodukte in der Ernährung des Menschen.* Volkswirtschaftlicher Verlag GmbH Kempten, Verlag Th. Mann OHG, Hildesheim, 1974.
7. Roy R N, Chance G W, Radde I C, Hill D E, Willis D M & Sheepers J. Late hyponatremia in very low birthweight infants (<1.3 kg). *Pediatr Res* 10: 526, 1976.
8. Ross B, Cowett R M & Oh W. Renal functions in low birth weight infants during the first two months of life. *Pediatr Res* 11: 1162, 1977.

Acta Pædiatr Scand 68 443–444 1979

SHORT COMMUNICATION

POLYCYSTIC KIDNEY DISEASE OF PERINATAL TYPE

Polycystic kidney disease of childhood is a recessively inherited heterogenous entity associated with a specific hepatic lesion (2 7) The most familiar and accepted form is the perinatal type of polycystic kidney disease of childhood (PPKD) Larroche calculated a PPKD frequency of 1 3/10³ child autopsies (7) We describe nine cases of PPKD in our child autopsy material

The study was based on hospital and autopsy records of five girls and four boys from seven different families with PPKD diagnosed and treated in our hospital during the years 1965–1977 We encountered no other types of polycystic kidney disease of childhood in our child autopsy material The mothers had a total of 29 pregnancies and 22 children including these newborns Of these 22 children 13 had clinically obvious PPKD but four died in other hospitals or before the year 1965 and were thus excluded from the study

During the time of the survey 1 524 child autopsies were performed Thus the frequency of PPKD was 5 9/10³ autopsies The incidence was 13 3/10 births

The gestational age ranged from 33 weeks to 40 weeks Four of these newborns were delivered by breech presentation Birth weight ranged from 1 900 to 4 140 g (Table 1) Oligo hydramnios was found in all but one of the cases in which the amount of amniotic fluid was recorded The placenta was large in seven cases the mean placental/fetal ratio being 31% and the range from 20 to 47% (Table 1) Survival time ranged from one to 34 hours and only one child lived over 24 hours

The lungs were atelectatic with haemorrhages Mild lung hypoplasia was found in two cases Other congenital malformations included talipes equinovarus in two cases mild hydrocephalus atrial septal defect and hypoplastic adrenal glands with a total weight of 2 g

Histologically the typical liver and renal findings were observed in all cases In six cases the pancreas specimen was available It showed dense periductal and lobular fibrosis the degree of fibrosis varying little from case to case (Table 1) The placenta was histologically immature The histological picture was similar to that in congenital nephrosis of the Finnish type (6)

The incidence of PPKD seems to be high in our material and is similar to the incidence of

Table 1 *Clinical and pathological findings of children with PPKD*

Case	Family	Sex	Weight (g)	Weight of kidneys (g)	Fibrosis of pancreas	Placental/fetal ratio (%)	Abnormal placental histology
1	I	F	1 900	93	+	4[illegible]	Not known
	I	F	040	136	+	47	+
3	II	M	3 370	343	Not known	[illegible]1	+
4	II	M	4 140	64	+	0	+
5	III	F	3 190	400	Not known	36	+
6	IV	F	3 050	150	+	34	Not known
	V	F	760	190	Not known	[illegible]7	Not known
8	VI	M	600	255	+	[illegible]5	+
9	VII	F	3 140	63	+	27	Not known

congenital nephrosis of the Finnish type in Finland (4) We found large placenta in our series and the same finding has been reported in the congenital nephrosis too Thus it seems that congenital renal diseases can cause large placenta Furthermore the breech presentation can be caused by big renal masses and the large placenta which blocks the turning of the fetus to the vertex position

As in earlier studies the segregation was compatible with autosomal recessive inheritance (2 8) Therefore in genetic counseling of PPKD it is important to tell parents that only future sibs may be affected In these families fetal sonography is indicated since the polycystic kidneys of the fetus sometimes can be recognized early enough to allow the parents to elect therapeutic abortion (1 3) It has also been reported that the dysplastic kidneys excrete excessive amounts of alpha fetoprotein which can be found in amniotic fluid (5)

M Uhari
Department of Paediatrics
University of Oulu
90220 Oulu 22
Finland

Riitta Herva
Department of Pathology
University of Oulu
90220 Oulu 22
Finland

REFERENCES

1 Bartley J A Golbus M S Filly R A & Hall J B D Prenatal diagnosis of dysplastic kidney disease *Clin Genet 11* 375 1977
2 Blyth H & Ockenden B G Polycystic disease of kidneys and liver presenting in childhood *J Med Genet 8* 257 1971
3 Garret W J Grunwald G & Robinson D E Prenatal diagnosis of fetal polycystic kidney by ultrasound *Aust NZ J Obstet Gynecol 10 1* 1970
4 Huttunen N P Congenital nephrotic syndrome of Finnish type Study of 75 patients *Arch Dis Child 51* 344 1976
5 Kaffe S Rose J S Godmilow L Walker B A Kerenyi T Beratis N Reyes P & Hirschhorn K Prenatal diagnosis of renal anomalies *Am J Med Genet 1* 241 1977
6 Kouvalainen K Hjelt L & Hallman N Placenta in congenital nephrotic syndrome *Ann Paediatr Fenn 8* 181 1962
7 Larroche J -C *Developmental pathologies of the neonate* Excerpta Medica Amsterdam 1977 p 96
8 Lundin P M & Olow I Polycystic kidneys in newborns infants and children A clinical and pathological study *Acta Paediatr Scand 50* 185 1961

CASE REPORT

PARTIAL TRISOMY 15 AND INTRACTABLE SEIZURES

K TAYSI D C DEVIVO and G S SEKHON

From the Edward Mallinckrodt Department of Pediatrics and the Department of Neurology and Neurosurgery (Neurology) Washington University School of Medicine and the Divisions of Medical Genetics and Neurology St Louis Children s Hospital St Louis Missouri USA

ABSTRACT Taysi K DeVivo D C and Sekhon G S (The Edward Mallinckrodt Department of Pediatrics and the Department of Neurology and Neurosurgery (Neurology) Washington University School of Medicine and the Divisions of Medical Genetics and Neurology St Louis Children s Hospital St Louis Missouri USA) Partial trisomy 15 and intractable seizures Acta Paediatr Scand 68 445 1979 —The clinical and cytogenetic findings of a case with partial trisomy of chromosome 15 are presented Dysmorphic features of this newly recognized syndrome are too nonspecific and mild to permit the physician to make clinical diagnosis or to consider obtaining cytogenetic studies in eary life However the present case indicates that seizures represent an important part of the clinical presentation of this rare chromosomal syndrome in addition to severe mental retardation

KEY WORDS Partial trisomy chromosome 15 seizures

Twenty-one cases of partial trisomy of chromosome No 15 have been reported in the cytogenetic literature since the initial description by Webb and colleagues in 1971 (7) The clinical features of this abnormality are not sufficiently distinctive to permit the physician to make the diagnosis clinically or to encourage one to obtain cytogenetic studies Consequently the majority of patients have not been diagnosed until they reach school age We uncovered another case of partial trisomy involving the long arm of chromosome No 15 while evaluating a young girl with intractable seizures profound psychomotor retardation and dysmorphic features The purpose of this report is to call attention to the fact that seizures represented an important part of the clinical picture in our patient and in approximately 50% of the reported cases (1–6 8)

CASE REPORT

The patient an 8 year-old black female was born to an 18 year-old primigravida There was no known exposure to drugs alcohol or other known teratogens during the uncomplicated pregnancy The infant was born at term by vaginal delivery in breech presentation after an uncomplicated labor The birth weight was 3 60 g and the neonatal period was normal However she was irritable and fed poorly She suffered a grand mal seizure at ? months of age Since then she has had recurrent grand mal seizures with variable frequency 1–2 per day to none for several months

Her development has been delayed She sat without support at 1 year walked clumsily at 1½ years was toilet trained at 2½ years and started to use simple sentences at 3 years of age There has been no remarkable developmental progress since then She has been evaluated extensively for the seizures which became progressively resistant to various medications including phenobarbital phenytoin carbamazepine and clonazepam in various doses and combinations She was admitted to St Louis Children s Hospital for further evaluation and treatment with a ketogenic diet While on the ketogenic diet for one year the seizure frequency decreased approximately 90% Recent addition of dipropylacetic acid to the treatment regimen has effected further control of the seizures

The family history is negative for birth defects mental retardation and seizures Her two younger siblings and both parents are healthy There is no consanguinity

The physical examination revealed a hyperactive mentally retarded girl with an ataxic gait Her height weight

Abbreviations GTG=G banding of chromosomes by trypsin using Giemsa QFQ=Q banding of chromosomes by fluorescence using quinacrine RHA=R banding of chromosomes by heating using acridine orange



Fig. 1. Patient at 8 years of age.

and head circumference were below the third percentile for age. Certain dysmorphic features were noted, including frontal bossing, decreased bifrontal diameter, flat occiput, prominent supraorbital ridges, heavy eyebrows, synophrys, telecanthus (inner canthal distance 3.4 cm), slight antimongoloid slanting of the palpebral fissures, bilateral epicanthal folds, slightly low set and posteriorly rotated ears with prominent antihelix and high arched palate (Fig. 1). There was bilateral clinodactyly of the 5th fingers and mild syndactyly between the second and third toes bilaterally. A small umbilical hernia also was noted. The dermatoglyphic analysis was unremarkable. Mental and motor retardation and poor motor coordination characterized the neurological findings.

Psychometric evaluation revealed the following results: DQ 20 (Cattel), SQ 38 (Vineland) and IQ 48 (Verbal Language). Normal laboratory studies included a complete blood count, electrolytes, BUN, glucose, Ca, Ph, Mg, uric acid, creatinine, alkaline phosphatase, serum proteins, urine amino acids, and thyroid function tests. A skull X ray, cerebral radionuclide angiogram and computed tomography were normal and the bone age was commensurate with the chronological age. The severely abnormal EEG showed multifocal independent spikes and nonfocal bursts of spike and slow waves superimposed upon a slow background.

CYTOGENETIC STUDIES

A chromosome analysis was performed on peripheral blood cultures of the patient and both parents. GTG, QFQ and RHA banding

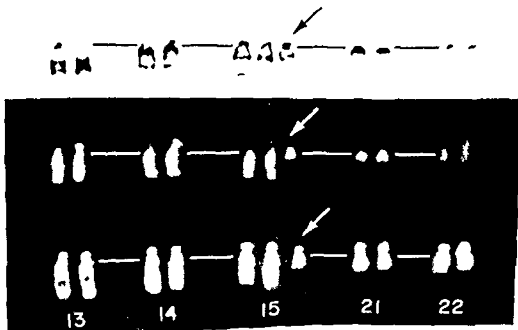


Fig. 2. In descending order GTG, QFQ and RHA banded partial karyotypes of the patient with extra No. 15 chromosome. The break occurred in band 2 of region 2 of the long arm of the extra chromosome 15.

techniques were employed in evaluating the patient s chromosomes

A small extra acrocentric chromosome was found in each of the patient s cells examined Partial GTG QFQ and RHA banded karyotypes are shown in Fig 2 Comparing different karyotypic banding patterns allowed us to identify the extra chromosome as No 15 with a deletion from band 22 of the long arm The patient was therefore trisomic for the pter→q?2 segment of chromosome No 15 47 XX + 15del(15) (q22)

Chromosome studies from both parents yielded normal karyotypes with GTG banding

DISCUSSION

The patient had intractable grand mal seizures severe psychomotor retardation and dysmorphic features None of these findings were sufficient to encourage the clinicians to request a karyotype analysis until she was 8 years old In reviewing the previously published cases with partial trisomy of the proximal long arm of chromosome 15 the main clinical features appeared to be mental retardation short stature microcephaly hypotonia seizure activity and certain dysmorphic features The latter included widely spaced heavy eyebrows telecanthus epicanthus strabismus antimongoloid slant low set posteriorly rotated ears large mouth full lips and high arched palate Scoliosis mild syndactyly of the toes and clinodactyly of the 5th fingers were variable features Nine patients including the present case had seizures of varying severity including myoclonic and grand mal types Our case shared most of these findings which in aggregate are rather nonspecific However the present case would suggest that karyotype analysis with different banding techniques be performed early in the evaluation of mental retardation associated with seizure disorders and somatic dysmorphia Results of karyotype analysis would be of potential diagnostic and counseling benefit

This report also illustrates the difficulty encountered in the identification of a small extra chromosome with few landmarks in the absence of a parental chromosome abnormality The proper diagnosis of the extra chromosome in this patient was achieved only after the employment of several banding techniques

ACKNOWLEDGEMENTS

This work was supported in part by the Ranken Jordan Trust Fund for the Crippling Diseases of Children and by USPHS grant RR 00036 from the Division of Research Resources

REFERENCES

1 Centerwall W R & Morris J P Partial D 15 trisomy *Hum Hered* 25 44? 1975
2 Cohen M M Ornoy A Rosenmann A & Kohn G An inherited translocation t(4 15)(p16 q??) leading to two cases of partial trisomy 15 *Ann Genet 18* 99 1975
3 Crandall B F Muller H M & Bass H N Partial Trisomy of chromosome number 15 identified by trypsin Giemsa banding *Am J Ment Def 77* 571 1973
4 Mankinen C B Holt J G & Sears J W Partial trisomy 15 in a young girl *Cl n Genet 10* ?7 1976
5 Power M M Barry R G Cannon D E & Masterson J G Familial partial trisomy 15 *Ann Genet 20* 159 1977
6 Watson E J & Gordon R R A case of partial trisomy 15 *J Med Genet 11* 400 1974
7 Webb G C Garson O M Robson M K & Pitt D B A partial D trisomy/normal mosaic female *J Med Genet 8* 5?2 1971
8 Wurster Hill D H & Hoefnagel D Banding identification of partial trisomy 15 and of 8/‿1 translocation *J Ment Def c Res* 18 139 1974

Submitted Sept 1? 1978
Accepted Oct 18 1978

(K T) Department of Pediatrics
Division of Medical Genetics
St Louis Children s Hospital
500 So Kingshighway
P O Box 14871
St Louis Missouri 63178
USA



Fig. 1. Patient at 8 years of age.

and head circumference were below the third percentile for age. Certain dysmorphic features were noted, including frontal bossing, decreased bifrontal diameter, flat occiput, prominent supraorbital ridges, heavy eyebrows, synophrys, telecanthus (inner canthal distance 3.4 cm), slight antimongoloid slanting of the palpebral fissures, bilateral epicanthal folds, slightly low set and posteriorly rotated ears with prominent antihelix and high arched palate (Fig. 1). There was bilateral clinodactyly of the 5th fingers and mild syndactyly between the second and third toes bilaterally. A small umbilical hernia also was noted. The dermatoglyphic analysis was unremarkable. Mental and motor retardation and poor motor coordination characterized the neurological findings.

Psychometric evaluation revealed the following results: DQ 70 (Cattel), SQ 38 (Vineland) and LQ 48 (Verbal Language). Normal laboratory studies included a complete blood count, electrolytes, BUN, glucose, Ca, Ph, Mg, uric acid, creatinine, alkaline phosphatase, serum proteins, urine amino acids, and thyroid function tests. A skull X ray, cerebral radionuclide angiogram and computed tomography were normal and the bone age was commensurate with the chronological age. The severely abnormal EEG showed multifocal independent spikes and nonfocal bursts of spike and slow waves superimposed upon a slow background.

CYTOGENETIC STUDIES

A chromosome analysis was performed on peripheral blood cultures of the patient and both parents. *GTG*, *QFQ* and *RHA* banding

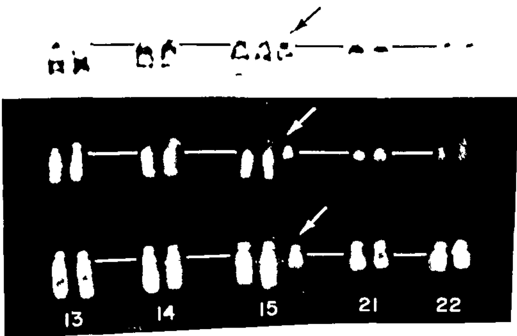


Fig. 2. In descending order GTG, QFQ and RHA banded partial karyotypes of the patient with extra No. 15 chromosome. The break occurred in band 2 of region 2 of the long arm of the extra chromosome 15.

CASE REPORT

THE COFFIN SIRIS SYNDROME

A SCHINZEL

From the Department of Medical Genetics University of Zurich Zurich Swit erland

ABSTRACT Schinzel A (Department of Medical Genetics University of Zurich Switzer land) The Coffin Siris syndrome Acta Paediatr Scand 68 449, 1979 —A 14 month-old female with the Coffin Siris syndrome is described Typical features included underweight at birth growth retardation microcephaly profound mental retardation severe hypotonia with lax joints feeding difficulties and frequent respiratory tract infections sparce scalp hair small nose epicanthic folds a prominent philtrum and full lips a congenital heart defect hypoplasia or aplasia of the distal phalanges of digits 2–5 and the corresponding nails especially of the fifth fingers and toes and aplasia of the middle phalanges of the little fingers and the second and fifth toes severe delay in bone maturation The proposita also showed hypoplasia of the lateral portions of both clavicles Inheritance of the Coffin Siris syndrome is possibly autosomal recessive

KEY WORDS Coffin Siris syndrome autosomal recessive inheritance phalangeal hypopla sia

In 1970 Coffin & Siris (3) described three un related patients with a distinct pattern of mal formations and severe mental retardation Subsequently only eight further cases have been reported (1 2 5 6) The present paper reports a further case of the Coffin Siris syndrome

CASE REPORT

Case history

The proposita was the product of the second pregnancy of a ?1 year-old mother and a 30-year-old father Family history was positive for diabetes mellitus and eczema there was no *consanguinity* The mother has never had any kind of epileptic episodes and never has taken any antiepileptic drugs The 2½ year old son is healthy and normal He was delivered by cesarean section because of a narrow pelvis of the mother his birthweight was 3 ?50 g

During the entire pregnancy with *the proposita* the mother suffered from abdominal pain and frequent vomit ing A one-day bleeding occurred in the 16th week At the end of the sixth month intrauterine growth retardation was first detected Repeated human placental lactogen blood levels and estriol values in the urine of the mother were normal At the 40th week of gestation cesarean section was performed because of a pathologic cardio- tocographic curve breech presentation and previous section The newborn was given artificial ventilation with oxygen for the first ten minutes Apgar scores were 6 9 and 9 after 1 5 and 10 minutes respectively

Clinical examination at birth disclosed severe muscular hypotonia and virtually complete lack of spontaneous movements short eyelids a hoarse cry a small anterior fontanel redundant nuchal skinfolds moderate hyper trichosis and hypoplasia or aplasia of the distal phal anges of the second through fifth fingers and toes Meas urements were 46 cm for length (10th percentile) 2 430 g for weight and 31 cm for head circumference (both below 10th percentile) An opthalmologic examination disclosed normal bulbi and fundi The proband s neonatal course was marked by failure to thrive poor sucking requiring intermittent tube feeding repeated cyanotic and apneic spells during and after feeding severe delay in psycho- motor development episodes of profuse sweating and frequent respiratory infections which necessitated five hospitalizations during the first 15 months of life A sys tolic murmur with its maximum at the left sternal border was first noticed at five weeks of age Neurologically severe muscular hypotonia persisted Moro and grasping reflexes were absent and spontaneous movements con tinued to be very poor as were tendon reflexes She started to fix with her eyes for a short span at 3½ months of age At that time intermittent convergent squint and intermittent horizontal nystagmus were noticed The first smile was observed at six months

At clinical examination at 14 months of age the pro posita presented as a microcephalic and severely hypo- tonic physically and mentally retarded female infant with multiple *dysmorphic* stigmata (Figs 1–?) Length (68 9 cm) weight (6 35 g) and head circumference (41 cm) were

Acta Pædiatr Scand 68 449–452 1979

CASE REPORT

THE COFFIN SIRIS SYNDROME

A SCHINZEL

From the Department of Medical Genetics University of Zurich Zurich Switzerland

ABSTRACT Schinzel A (Department of Medical Genetics University of Zurich Switzerland) The Coffin Siris syndrome Acta Paediatr Scand 68 449 1979.—A 14-month-old female with the Coffin Siris syndrome is described Typical features included underweight at birth growth retardation microcephaly profound mental retardation severe hypotonia with lax joints feeding difficulties and frequent respiratory tract infections, sparce scalp hair small nose epicanthic folds a prominent philtrum and full lips a congenital heart defect hypoplasia or aplasia of the distal phalanges of digits 2–5 and the corresponding nails especially of the fifth fingers and toes and aplasia of the middle phalanges of the little fingers and the second and fifth toes severe delay in bone maturation The proposita also showed hypoplasia of the lateral portions of both clavicles Inheritance of the Coffin Siris syndrome is possibly autosomal recessive

KEY WORDS Coffin Siris syndrome autosomal recessive inheritance phalangeal hypoplasia

In 1970 Coffin & Siris (3) described three unrelated patients with a distinct pattern of malformations and severe mental retardation Subsequently only eight further cases have been reported (1 2 5 6) The present paper reports a further case of the Coffin Siris syndrome

CASE REPORT

Case history

The proposita was the product of the second pregnancy of a ?1 year-old mother and a 30-year-old father Family history was positive for diabetes mellitus and eczema there was no consanguinity The mother has never had any kind of epileptic episodes and never has taken any antiepileptic drugs The 2½ year-old son is healthy and normal He was delivered by cesarean section because of a narrow pelvis of the mother his birthweight was 3 ?50 g

During the entire pregnancy with the proposita the mother suffered from abdominal pain and frequent vomiting A one day bleeding occurred in the 16th week At the end of the sixth month intrauterine growth retardation was first detected Repeated human placental lactogen blood levels and estriol values in the urine of the mother were normal At the 40th week of gestation cesarean section was performed because of a pathologic cardiotocographic curve breech presentation and previous section The newborn was given artificial ventilation with oxygen for the first ten minutes Apgar scores were 6 9 and 9 after 1 5 and 10 minutes respectively

Clinical examination at birth disclosed severe muscular hypotonia and virtually complete lack of spontaneous movements short eyelid a hoarse cry a small anterior fontanel redundant nuchal skinfolds moderate hypertrichosis and hypoplasia or aplasia of the distal phalanges of the second through fifth fingers and toes Measurements were 46 cm for length (10th percentile) ~430 g for weight and 31 cm for head circumference (both below 10th percentile) An opthalmologic examination disclosed normal bulbi and fundi The proband's neonatal course was marked by failure to thrive poor sucking requiring intermittent tube feeding repeated cyanotic and apneic spells during and after feeding severe delay in psychomotor development episodes of profuse sweating and frequent respiratory infections which necessitated five hospitalizations during the first 15 months of life A systolic murmur with its maximum at the left sternal border was first noticed at five weeks of age Neurologically severe muscular hypotonia persisted Moro and grasping reflexes were absent and spontaneous movements continued to be very poor as were tendon reflexes She started to fix with her eyes for a short span at 3½ months of age At that time intermittent convergent squint and intermittent horizontal nystagmus were noticed The first smile was observed at six months

At clinical examination at 14 months of age the proposita presented as a microcephalic and severely hypotonic physically and mentally retarded female infant with multiple dysmorphic stigmata (Figs 1–2) Length (68 9 cm) weight (6 35 g) and head circumference (41 cm) were

Table 1 *Main findings in twelve cases of the Coffin Siris syndrome as compared to their incidence in the fetal hydantoin syndrome*

	References		Present case	Fetal hydantoin syndrome (4)
	2 3 5 6	1		
Birthweight below 3rd percentile	6/10	–	+	13/14
Postnatal growth retardation	9/9	–	+	14/15
Microcephaly	9/10	–	+	13/15
Mental retardation (IQ below 85)	9/9	+	+	9/13
Muscular hypotonia	10/10	+	+	0/15
Retarded bone maturation	5/6	–	+	Not mentioned
Feeding difficulties	9/10	–	+	n m
Recurrent respiratory infections	5/10	–	+	n m
Hypertrichosis	9/10	?	+	n m
Sparce scalp hair	8/9	?	+	n m
Facial dysmorphisms	10/10	+	+	6/15
Full lips	8/9	–	+	n m
Hypoplasia of terminal phalanges and nails	10/10	+	+	4/15
Hyperextendible joints	10/10	+	+	n m
Cleft palate	?/10	–	–	1/15
Congenital heart defect	4/10	–	+	3/15
Dandy Walker malformation in autopsied cases	?/3	0	0	0

and the left index finger were absent and those of the other fingers except for the thumbs were hypoplastic most pronounced for the right index and the left middle finger (Fig) This nail hypoplasia was associated with shortening of the distal phalanges of the corresponding fingers The thumbs were mostly in flexion and opposition there was clinodactyly of the little fingers The feet were in adduction and calcaneo-valgus position The nails of the second and fifth toes were absent and the nails of the third and fourth toes were hypoplastic Tendon reflexes were very week The proposita reacted to sounds and rarely smiled but she did not turn around or sit up Grasping was very poor and she was barely able to lift her head A Denver development test revealed a developmental age of 4–5 months

The further course was marked by repeated respiratory infections which were accompanied with mild cardiac failure and persisting severe mental retardation Presently at 5/12 years of age she is still unable to sit up and has no speech

X ray examinations

On *chest films* performed at 14 months of age (Fig 3) the heart was enlarged to the left and lung vascularity was increased A pneumonia was seen in the left lower lobe behind the heart There was striking hypoplasia and superiorly convex curving of the lateral portions of the clavicles Radiographs of *both hands* at three days of age (Fig 4) revealed no ossification of the distal phalanges of both second and fifth fingers and the middle phalanx of the little fingers and hypoplasia of the distal phalanges of the third and fourth fingers At 14 months the findings were similar and bone age was less than three months Radiographs of *both feet* at three days of age (Fig 5) showed hindfoot valgus and absence or hypoplasia of the middle and distal phalanges of toes –5 Skull and pelvic films at 14 months of age were considered normal

Other examinations

An E K G showed right ventricular hypertrophy In combination with the clinical findings and the chest films a



Fig 5 Radiographs of both feet 3 days of age No ossification of any distal phalanges except for the big toes nor of the middle phalanges of the second and fifth toes hypoplasia of the middle phalanges of the third and fourth toes



Fig 1 Head of the proposita aged 14 months Sparce scalp hair short nose with broad bridge bilateral epicanthic folds convergent squint of both eyes prominent philtrum and lips

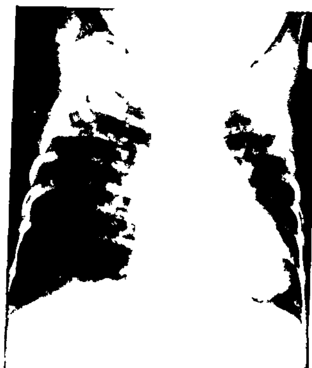


Fig 3 Chest films from the proposita at 14 months of age Enlarged heart increased lung vascularity left lower lobe consolidation and hypoplasia of the lateral portions of both clavicles

below the 3rd percentile Scalp hair was sparce The facies was small and narrow and exhibited a broad and short nose narrow palpebral fissures (on the left side more than on the right) bilateral epicanthal folds convergent strabismus of both eyes normal fundi full philtrum and lips a small mouth and a rather large plump tongue and a misshapen left ear There was cutis laxa over the nuchal area and a chronic eczema of the retroauricular nuchal and dorsal upper thoracic regions and mild hypertrichosis of the back and dorsal upper arms Cardiac auscultation disclosed a 4/6 systolic murmur over the entire heart and the back the liver was palpable 2 3 cm but the spleen was not palpable The abdominal muscles were hypoplastic with diastasis of the rectus muscles No hernias were present genitalia were normal there was a precoccygeal dimple All large and small joints were hyperextensible The nails of both little fingers

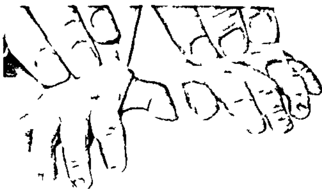


Fig 2 Both hands of the proposita aged 14 months Hypoplastic terminal phalanges of fingers 2–5 with absent nails on little fingers and hypoplastic nails on fingers 2–4 (especially both index fingers and the left middle finger) Clinodactyly of both little fingers



Fig 4 Radiographs of both hands 3 days of age No ossification of distal phalanges of the second and fifth fingers and of middle phalanges of the fifth fingers hypoplasia of distal phalanges of the third and fourth fingers

Table 1 *Main findings in twelve cases of the Coffin Siris syndrome as compared to their incidence in the fetal hydantoin syndrome*

	References		Present case	Fetal hydantoin syndrome (4)
	2 3 5 6	1		
Birthweight below 3rd percentile	6/10	–	+	13/14
Postnatal growth retardation	9/9	–	+	14/15
Microcephaly	9/10	–	+	13/15
Mental retardation (IQ below 85)	9/9	+	+	9/13
Muscular hypotonia	10/10	+	+	0/15
Retarded bone maturation	5/6	–	+	Not mentioned
Feeding difficulties	9/10	–	+	n m
Recurrent respiratory infections	5/10	–	+	n m
Hypertrichosis	9/10	?	+	n m
Sparce scalp hair	8/9	?	+	n m
Facial dysmorphisms	10/10	+	+	6/15
Full lips	8/9	–	+	n m
Hypoplasia of terminal phalanges and nails	10/10	+	+	4/15
Hyperextendible joints	10/10	+	+	n m
Cleft palate	2/10	–	–	1/15
Congenital heart defect	4/10	–	+	3/15
Dandy Walker malformation in autopsied cases	/3	0	0	0

and the left index finger were absent and those of the other fingers except for the thumbs were hypoplastic most pronounced for the right index and the left middle finger (Fig 2) This nail hypoplasia was associated with shortening of the distal phalanges of the corresponding fingers The thumbs were mostly in flexion and opposition there was clinodactyly of the little fingers The feet were in adduction and calcaneo-valgus position The nails of the second and fifth toes were absent and the nails of the third and fourth toes were hypoplastic Tendon reflexes were very week The proposita reacted to sounds and rarely smiled but she did not turn around or sit up Grasping was very poor and she was barely able to lift her head A Denver development test revealed a developmental age of 4–5 months

The further course was marked by repeated respiratory infections which were accompanied with mild cardiac failure and persisting severe mental retardation Presently at 5/12 years of age she is still unable to sit up and has no speech

X-ray examinations

On *chest films* performed at 14 months of age (Fig 3) the heart was enlarged to the left and lung vascularity was increased A pneumonia was seen in the left lower lobe behind the heart There was sinking hypoplasia and superiorly convex curving of the lateral portions of the clavicles Radiographs of *both hands* at three days of age (Fig 4) revealed no ossification of the distal phalanges of both second and fifth fingers and the middle phalanx of the little fingers and hypoplasia of the distal phalanges of the third and fourth fingers At 14 months the findings were similar and bone age was less than three months Radiographs of *both feet* at three days of age (Fig 5) showed hindfoot valgus and absence or hypoplasia of the middle and distal phalanges of toes 2–5 Skull and pelvic films at 14 months of age were considered normal

Other examinations

An E K G showed right ventricular hypertrophy In combination with the clinical findings and the chest films a

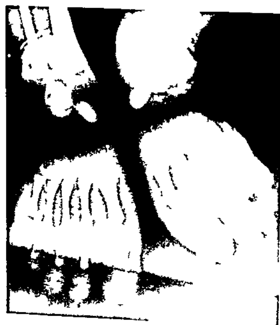


Fig 5 Radiographs of both feet 3 days of age No ossification of any distal phalanges except for the big toes nor of the middle phalanges of the second and fifth toes hypoplasia of the middle phalanges of the third and fourth toes

ventricular septal defect possibly combined with a mild pulmonic stenosis seemed most likely No cardiac catheterization was performed

Tests for thyroid function gave normal results Toxoplasmosis titer was negative A banded chromosome examination disclosed a normal 46 XX karyotype

DISCUSSION

The proposita is a characteristic case of the Coffin Siris syndrome (Table 1) She revealed all the pertinent features of this syndrome including pre and postnatal growth deficit severe delay of bone maturation microcephaly muscular hypotonia mental retardation hypoplasia of distal phalanges and nails sparce scalp hair hypertrichosis repeated respiratory tract infections and a characteristic facies with short and broad nose epicanthal folds and prominence of philtrum and lips Less consistent findings of the Coffin Siris syndrome are congenital heart defects and cleft palate the latter was not found in the proposita The Dandy Walker malformation was observed in the only two autopsied cases (1 6) Hypoplasia of the distal portions of the clavicles has not been described in previous cases but it may have been overlooked

One case showed an incomplete clinical picture (1) (see Table 1) Presently the knowledge of the variability of the clinical findings in the Coffin Siris syndrome is too small to allow to be sure that this case really represents the same syndrome

The fetal hydantoin syndrome shares some clinical features with the Coffin Siris syndrome notably distal limb hypoplasia pre and postnatal growth retardation microcephaly mental retardation cleft palate and congenital heart defects (4) (see Table 1) However differentiation of these two clinical entities by pregnancy history (intake of hydantoin) as well as clinical findings will generally not be difficult In the Coffin Siris syndrome in contrast to the fetal hydantoin syndrome growth and mental retardation is usually more severe scalp hair is sparce and hypoplasia of the distal phalanges is most severe in the fifth and second digits and not found in the thumbs and big toes In addition the pattern of facial dysmorphisms is different in the fetal hydantoin syndrome a prominent forehead synophris depressed nasal bridge and a strikingly short upturned nose are the most outstanding features and a prominent philtrum is sometimes found whereas in the Coffin Siris syndrome full lips and a prominent philtrum are predominant and the nose is normal or slightly shortened

Karyotypes have been normal in all cases so far reported (2 3 5 present report) Although there was no instance of parental consanguinity occurrence of the Coffin Siris syndrome in two sibs of different sex (2) out of a total of twelve reported cases indicates that inheritance might be autosomal recessive

ACKNOWLEDGEMENTS

The author wishes to thank Prof A Giedion (Division of Pediatric Radiology Department of Pediatrics University of Zurich) for valuable comments and Prof W Schmid (Department of Medical Genetics University of Zurich) for the karyotype examination and for a review of the manuscript

REFERENCES

1 Bartsokas C S & Tsiantos A K Mental retardation with absent fifth fingernail and terminal phalanx *Am J Dis Child 120* 493 1970
2 Carey J C & Hall B D The Coffin Siris syndrome *Am J Dis Child 132* 667 1978
3 Coffin G S & Siris E Mental retardation with absent fifth fingernail and terminal phalanx *Am J Dis Child 119* 433 1970
4 Hanson J W Myrianthopoulos N C Sedwick Harvey M A & Smith D W Risk to the offspring of women treated with hydantoin anticonvulsants with emphasis on the fetal hydantoin syndrome *J Pediatr 89* 662 1976
5 Tunnessen W W McMillan J A & Levin M B The Coffin Siris syndrome *Am J Dis Child 132* 393 1978
6 Weiswasser W H Hall B D Delavan G W & Smith D W Coffin Siris syndrome *Am J Dis Child 125* 838 1973

Submitted Aug 12 1978
Accepted Oct 27 1978

(A S) Department of Medical Genetics
University of Zurich
Steinwiesstr 75
CH 8032 Zurich
Switzerland

Acta Pædiatr Scand 68 453–458 1979

CASE REPORT

REGIONAL DEFICIENCY OF SECRETORY IgA IN A PATIENT WITH COMBINED IMMUNODEFICIENCY OF THE ADA DEFICIENT TYPE

E C MOORE R J LAFFIN T TOMASI * R J PICKERING J RADL and H J MEUWISSEN

From the New York State Department of Health Birth Defects and Kidney Disease Institutes The Departments of Pediatrics and Microbiology Albany Medical College Albany New York the Department of Immunology Mayo Medical and Graduate School of Rochester Minnesota USA and the Institute for Experimental Gerontology TNO Rijswijk The Netherlands

ABSTRACT Moore E C Laffin R J Tomasi T Pickering R J Radl J and Meuwissen H J (Department of Pediatrics Albany Medical College Albany New York New York State Department of Health Birth Defects and Kidney Disease Institutes Department of Immunology Mayo Medical and Graduate School Rochester Minnesota USA and Institute for Experimental Gerontology TNO Rijswijk The Netherlands) Regional deficiency of secretory IgA in a patient with combined immunodeficiency of the ADA deficient type Acta Paediatr Scand 68 453 1979 —The IgA system in a patient with SCID and ADA deficiency showed heterogeneity Serum IgA and stool secretory IgA (SIgA) levels were normal but with altered κ/λ and A_1/A_2 subclass ratios IgA in saliva and urine was deficient Amounts of secretory component were normal Jejunal and rectal biopsies showed prominent lymphonodular hyperplasia but no cells containing IgA A normal serum IgA level therefore does not always predict an intact secretory IgA system

KEY WORDS IgA combined immunodeficiency disease adenosine deaminase deficiency

In almost all patients reported the presence or absence of serum IgA has correlated well with the presence or absence of secretory IgA (SIgA) Only rare patients have either possessed secretory IgA without serum IgA (4 17) or had normal serum IgA with deficient saliva IgA (2 4 15 16)

Our patient had severe combined immune deficiency disease (SCID) with normal IgA levels in serum and stool but was markedly deficient in salivary and urinary IgA Secretory component (SC) was readily detectable She was the first patient found lacking adenosine deaminase (ADA) (1 5 6 7)

CASE REPORT

This child had persistent pneumonia and candida infections from the second week of life After six months of age when the diagnosis of SCID was made she was nursed in a low pathogen environment where despite persistent interstitial pulmonary disease and intermittent oral candidiasis she did quite well for the next 1½ years Fetal thymus and liver transfer factor and maternal buffy coat cells (washed and irradiated) produced no significant improvement

There were no HLA compatible siblings but because the patient did not stimulate her mother s cells in mixed lymphocyte culture maternal marrow transplantation was attempted with re-establishment of B cell but not of T cell function The patient concurrently developed active cytomegalovirus infection with dissemination and died (7)

METHODS

All studies unless so stated were prior to marrow transplantation

Serum immunoglobulin (Ig) levels κ and λ light chain typing subclass typing and analysis for dimeric structure of serum IgA were performed according to published methods (3 11 12)

Supported by PHS Grant 5 RO1 AI 11717 and Clinical Studies Center Grant RR-00749

ventricular septal defect possibly combined with a mild pulmonic stenosis seemed most likely No cardiac catheterization was performed

Tests for thyroid function gave normal results Toxoplasmosis titer was negative A banded chromosome examination disclosed a normal 46 XX karyotype

DISCUSSION

The proposita is a characteristic case of the Coffin Siris syndrome (Table 1) She revealed all the pertinent features of this syndrome including pre and postnatal growth deficit severe delay of bone maturation microcephaly muscular hypotonia mental retardation hypoplasia of distal phalanges and nails sparce scalp hair hypertrichosis repeated respiratory tract infections and a characteristic facies with short and broad nose epicanthal folds and prominence of philtrum and lips Less consistent findings of the Coffin Siris syndrome are congenital heart defects and cleft palate the latter was not found in the proposita The Dandy Walker malformation was observed in the only two autopsied cases (1 6) Hypoplasia of the distal portions of the clavicles has not been described in previous cases but it may have been overlooked

One case showed an incomplete clinical picture (1) (see Table 1) Presently the knowledge of the variability of the clinical findings in the Coffin Siris syndrome is too small to allow to be sure that this case really represents the same syndrome

The fetal hydantoin syndrome shares some clinical features with the Coffin Siris syndrome notably distal limb hypoplasia pre and postnatal growth retardation microcephaly mental retardation cleft palate and congenital heart defects (4) (see Table 1) However differentiation of these two clinical entities by pregnancy history (intake of hydantoin) as well as clinical findings will generally not be difficult In the Coffin Siris syndrome in contrast to the fetal hydantoin syndrome growth and mental retardation is usually more severe, scalp hair is sparce and hypoplasia of the distal phalanges is most severe in the fifth and second digits and not found in the thumbs and big toes In addition the pattern of facial dysmorphisms is different in the fetal hydantoin syndrome a prominent forehead synophris depressed nasal bridge and a strikingly short upturned nose are the most outstanding features, and a prominent philtrum is sometimes found whereas in the Coffin Siris syndrome full lips and a prominent philtrum are predominant and the nose is normal or slightly shortened

Karyotypes have been normal in all cases so far reported (2 3 5 present report) Although there was no instance of parental consanguinity occurrence of the Coffin Siris syndrome in two sibs of different sex (2) out of a total of twelve reported cases indicates that inheritance might be autosomal recessive

ACKNOWLEDGEMENTS

The author wishes to thank Prof A Giedion (Division of Pediatric Radiology Department of Pediatrics University of Zurich) for valuable comments and Prof W Schmid (Department of Medical Genetics University of Zurich) for the karyotype examination and for a review of the manuscript

REFERENCES

1 Bartsokas C S & Tsiantos A K Mental retardation with absent fifth fingernail and terminal phalanx *Am J Dis Child 120* 493 1970

2 Carey J C & Hall B D The Coffin Siris syndrome *Am J Dis Child 132* 667 1978

3 Coffin G S & Siris E Mental retardation with absent fifth fingernail and terminal phalanx *Am J Dis Child 119* 433 1970

4 Hanson J W Mynanthopoulos N C Sedwick Harvey M A & Smith D W Risk to the offspring of women treated with hydantoin anticonvulsants with emphasis on the fetal hydantoin syndrome *J Pediatr 89* 662 1976

5 Tunnessen W W McMillan J A & Levin M B The Coffin Siris syndrome *Am J Dis Child 132* 393 1978

6 Weiswasser W H Hall B D Delavan G W & Smith D W Coffin Siris syndrome *Am J Dis Child 125* 838 1973

Submitted Aug 12 1978
Accepted Oct 27 1978

(A S) Department of Medical Genetics
University of Zurich
Steinwiesstr 75
CH 8032 Zurich
Switzerland

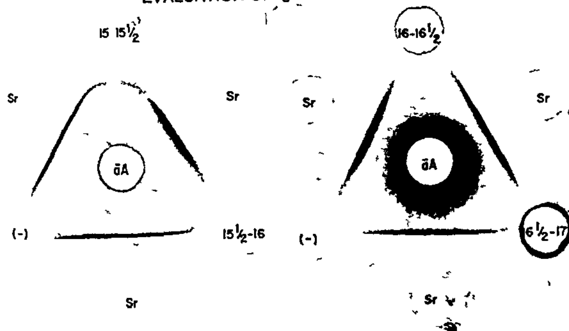


Fig Double diffusion with anti IgA antisera (aA in center well) *Sr* Serum diluted to a concentration of IgA of 10⁻⁷ mg (−) blank well Other wells contain saliva concentrated 40 times The numbers refer to the patient s age in months when the saliva was collected The decline in IgA levels with increasing age is evident

dominantly IgA κ type When marrow cells were stained with anti α chain antibody tagged with fluorescein isothiocyanate (FITC) in combination with anti κ chain antibody tagged with tetramethylrhodamine isothiocyanate (TRITC) 14α positive cells were found of which nine were also positive for κ When stained with anti-α chain FITC in combination with anti λ chain TRITC 16α positive cells were found none positive for λ

By double immunodiffusion IgA could be identified in saliva in trace amounts only when the saliva was concentrated at least 40 times and only before the 17th month of life (Figs 2 and 3) By radio immuno assay saliva IgA levels were less than 0 001 mg/dl (various samples) There was not enough material available to determine if this IgA was SIgA SC appeared quantitatively normal on double immunodiffusion Traces of IgG in saliva were also found during this period but IgM was not detected A lip biopsy in the 15th month showed no cells containing IgG IgM or IgA

IgA was easily detected in the patient s stools by double immunodiffusion in amounts grossly similar to those found in stools of five controls IgA levels by radio immuno assay were between 14 and 30 mg/dl in extracts of five patient stool samples whereas an age matched control stool sample contained 17 2 mg/dl (average of two measurements) This IgA was shown to be at least partly associated with SC by immunoelectrophoretic studies (Fig 3) and selective immunodiffusion IgM was not found in any of the patient s stools but they and two of five control stool samples contained traces of IgG

Radiologic study of the gut in the 15th month showed numerous small round filling defects jejunal and rectal biopsies showed these to represent lymphonodular aggregates Immunofluorescent staining of the specimens

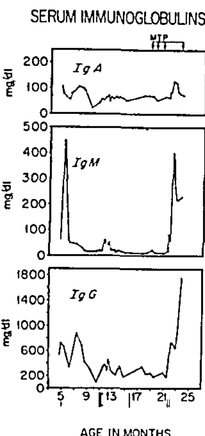


Fig 1 Immunoglobulin levels versus age Administration of gamma globulin or plasma is indicated by the vertical lines along the abscissa Arrows indicate marrow transplantation (MTP) The clear areas indicate the range of normal The IgM peak in the 5th month followed a series of immunizations (DPT typhoid)

Saliva stool and urine samples were kept at −30 C until studied Whole saliva was collected several times a week after stimulation with sterile lemon juice Specimens were filtered (1 2 μ pore size) Saliva samples pooled by month were concentrated by either in Amicon positive pressure ultrafiltration cell (retaining substances >30 000 daltons) or Lyphogel Polyacrylamide gel (Gelman Instrument Company)

Stool specimens were extracted by mixing with 2–3 volumes of normal saline with 2 4×10⁻⁴% merthiolate and 2 4×10⁻³% soybean trypsin inhibitor After centrifugation the supernatant was concentrated with Lyphogel to the original volume of the specimen Control samples were from normal children and patients of similar age with non infectious and non gastroenterological illnesses

Urine samples pooled by time intervals were centrifuged and the supernatant was concentrated by filtration through a collodion bag retaining substances >25 000 daltons

Igs and CS in stool extracts and in saliva concentrated to more than 100 fold were studied by double diffusion using mono specific antisera (Nordic Hyland) IgA levels in urine saliva and stool extracts were measured by radio immuno assay technique (8 9)

Stool specimens with adequate IgA levels were studied by immunoelectrophoresis using various anti SC antisera (Nordic and prepared by author (TT)) to see if the SC would migrate with the IgA indicating SIgA These specimens were also studied by an immunoselection technique in which anti-*free* SC antibody (Behring) was incorporated in the gel and the specimen reacted in double diffusion against anti IgA and an anti SC antibody (Nordic) capable of reacting with SC *either* free or in association with IgA

Specimens obtained by biopsy and autopsy tissues were frozen in liquid nitrogen and stained within two months with fluoresceinated antisera (Hyland) (10)

Marrow cells were examined for intracellular Igs by immunofluorescence techniques by Dr J Vossen (18)

RESULTS

Our patient s serum Ig levels were normal or elevated during her first months of life but after nine months IgG and IgM levels became subnormal (Fig 1) Serum IgA levels remained normal This IgA of subclass A_1 was largely IgA κ and migrated primarily cathodally on electrophoresis An antiserum specific for the conformational dimeric structure of IgA failed to precipitate IgA in the serum (14) Cytoplasmic immunofluorescence on marrow obtained at age 19 months identified a small number of IgA positive cells (Table 1) pre

Table 1 *Number of Ig containing cells in bone marrow as determined by immunofluorescence*

	Immunoglobulin						
	γ	α	μ	δ	κ	λ	κ/λ ratio
Patient	4	21	1	0	19	4	
Normal	122[b] (34–252)	109 (47–260)	60 (45–117)	0 9 (0–21)			1 65 0 81–2 44

a Number of Ig containing cells per 10³ immunocytes (lymphocytes or plasma cells)
b Geometric mean plus range

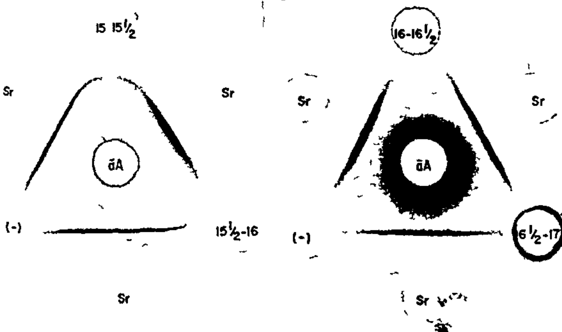


Fig 2 Double diffusion with anti IgA antisera (αA in center wells) *Sr* Serum diluted to a concentration of IgA of 10^{-4} mg (−) blank well Other wells contain saliva concentrated 40 times The numbers refer to the patient s age in months when the saliva was collected The decline in IgA levels with increasing age is evident

dominantly IgA κ type When marrow cells were stained with anti α chain antibody tagged with fluorescein isothiocyanate (FITC) in combination with anti κ chain antibody tagged with tetramethylrhodamine isothiocyanate (TRITC) 14α positive cells were found of which nine were also positive for κ When stained with anti-α chain FITC in combination with anti λ chain TRITC 16α positive cells were found none positive for λ

By double immunodiffusion IgA could be identified in saliva in trace amounts only when the saliva was concentrated at least 40 times and only before the 17th month of life (Figs 2 and 3) By radio immuno assay saliva IgA levels were less than 0 001 mg/dl (various samples) There was not enough material available to determine if this IgA was SIgA SC appeared quantitatively normal on double immunodiffusion Traces of IgG in saliva were also found during this period but IgM was not detected A lip biopsy in the 15th month showed no cells containing IgG IgM or IgA

IgA was easily detected in the patient s stools by double immunodiffusion in amounts grossly similar to those found in stools of five controls IgA levels by radio immuno assay were between 14 and 30 mg/dl in extracts of five patient stool samples whereas an age matched control stool sample contained 17 2 mg/dl (average of two measurements) This IgA was shown to be at least partly associated with SC by immunoelectrophoretic studies (Fig 3) and selective immunodiffusion IgM was not found in any of the patient s stools but they and two of five control stool samples contained traces of IgG

Radiologic study of the gut in the 15th month showed numerous small round filling defects jejunal and rectal biopsies showed these to represent lymphonodular aggregates Immunofluorescent staining of the specimens

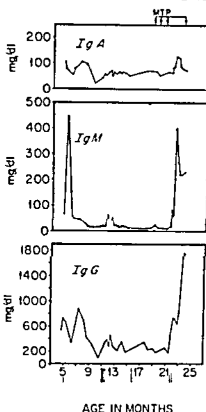


Fig 1 Immunoglobulin levels versus age Administration of gamma globulin or plasma is indicated by the vertical lines along the abscissa Arrows indicate marrow transplantation (MTP) The clear areas indicate the range of normal The IgM peak in the 5th month followed a series of immunizations (DPT typhoid)

Saliva stool and urine samples were kept at −30 C until studied Whole saliva was collected several times a week after stimulation with sterile lemon juice Specimens were filtered (1 2 μ pore size) Saliva samples pooled by month were concentrated by either an Amicon positive pressure ultrafiltration cell (retaining substances >30 000 daltons) or Lyphogel Polyacrylamide gel (Gelman Instrument Company)

Stool specimens were extracted by mixing with 2–3 volumes of normal saline with 2 4×10⁻⁶% merthiolate and 2 4×10⁻³% soybean trypsin inhibitor After centrifugation the supernatant was concentrated with Lyphogel to the original volume of the specimen Control samples were from normal children and patients of similar age with non infectious and non gastroenterological illnesses

Urine samples pooled by time intervals were centrifuged and the supernatant was concentrated by filtration through a collodion bag retaining substances >25 000 daltons

Igs and CS in stool extracts and in saliva concentrated to more than 100 fold were studied by double diffusion using mono specific antisera (Nordic Hyland) IgA levels in urine saliva and stool extracts were measured by radio immuno assay technique (8 9)

Stool specimens with adequate IgA levels were studied by immunoelectrophoresis using various anti SC antisera (*Nordic and prepared by author (TT)*) to see if the SC would migrate with the IgA indicating SIgA These specimens were also studied by an immunoselection technique in which anti-*free* SC antibody (Behring) was incorporated in the gel and the specimen reacted in double diffusion against anti IgA and an anti SC antibody (Nordic) capable of reacting with SC *either* free or in association with IgA

Specimens obtained by biopsy and autopsy tissues were frozen in liquid nitrogen and stained within two months with fluoresceinated antisera (Hyland) (10)

Marrow cells were examined for intracellular Igs by immunofluorescence techniques by Dr J Vossen (13)

RESULTS

Our patient s serum Ig levels were normal or elevated during her first months of life but after nine months IgG and IgM levels became subnormal (Fig 1) Serum IgA levels remained normal This IgA of subclass A_1 was largely IgA κ and migrated primarily cathodally on electrophoresis An antiserum specific for the conformational dimeric structure of IgA failed to precipitate IgA in the serum (14) Cytoplasmic immunofluorescence on marrow obtained at age 19 months identified a small number of IgA positive cells (Table 1) pre

Table 1 *Number of Ig containing cells in bone marrow as determined by immunofluorescence*

	Immunoglobulin γ	α	μ	δ	κ	λ	κ/λ ratio
Patient	4	21	1	0	19	4	
Normal	122[b] (34–252)	109 (47–260)	60 (45–117)	0 9 (0–21)			1 65 0 81–2 44

Number of Ig containing cells per 10³ immunocytes (lymphocytes or plasma cells)
[b] Geometric mean plus range

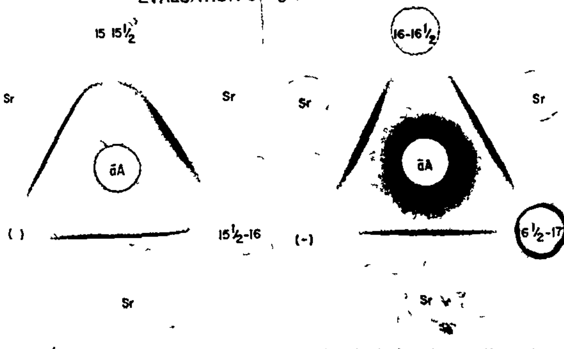


Fig 2 Double diffusion with anti IgA antisera (aA in center wells) *Sr* Serum diluted to a concentration of IgA of 10^{-7} mg (−) blank well Other wells contain saliva con entrated 40 times The numbers refer to the patient s age in months when the saliva was collected The decline in IgA levels with increasing age is evident

dominantly IgA κ type When marrow cells were stained with anti α chain antibody tagged with fluorescein isothiocyanate (FITC) in combination with anti κ chain antibody tagged with tetramethylrhodamine isothiocyanate (TRITC) 14α positive cells were found of which nine were also positive for κ When stained with anti-α chain FITC in combination with anti λ chain TRITC 16α positive cells were found none positive for λ

By double immunodiffusion IgA could be identified in saliva in trace amounts only when the saliva was concentrated at least 40 times and only before the 17th month of life (Figs 2 and 3) By radio immuno assay saliva IgA levels were less than 0 001 mg/dl (various samples) There was not enough material available to determine if this IgA was SIgA SC appeared quantitatively normal on double immunodiffusion Traces of IgG in saliva were also found during this period but IgM was not detected A lip biopsy in the 15th month showed no cells containing IgG IgM or IgA

IgA was easily detected in the patient s stools by double immunodiffusion in amounts grossly similar to those found in stools of five controls IgA levels by radio immuno assay were between 14 and 30 mg/dl in extracts of five patient stool samples whereas an age matched control stool sample contained 17 2 mg/dl (average of two measurements) This IgA was shown to be at least partly associated with SC by immunoelectrophoretic studies (Fig 3) and selective immunodiffusion IgM was not found in any of the patient s stools but they and two of five control stool samples contained traces of IgG

Radiologic study of the gut in the 15th month showed numerous small round filling defects jejunal and rectal biopsies showed these to represent lymphonodular aggregates Immunofluorescent staining of the specimens

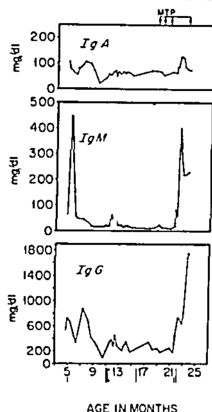


Fig 1 Immunoglobulin levels versus age Administration of gamma globulin or plasma is indicated by the vertical lines along the abscissa Arrows indicate marrow transplantation (MTP) The clear areas indicate the range of normal The IgM peak in the 5th month followed a series of immunizations (DPT typhoid)

Saliva stool and urine samples were kept at −30 C until studied Whole saliva was collected several times a week after stimulation with sterile lemon juice Specimens were filtered (1 2 μ pore size) Saliva samples pooled by month were concentrated by either an Amicon positive pressure ultrafiltration cell (retaining substances >30 000 daltons) or Lyphogel Polyacrylamide gel (Gelman Instrument Company)

Stool specimens were extracted by mixing with 2–3 volumes of normal saline with 2 4×10 $^{-6}$% merthiolate and 2 4×10 $^{-3}$% soybean trypsin inhibitor After centrifugation the supernatant was concentrated with Lyphogel to the original volume of the specimen Control samples were from normal children and patients of similar age with non infectious and non gastroenterological illnesses

Urine samples pooled by time intervals were centrifuged and the supernatant was concentrated by filtration through a collodion bag retaining substances >25 000 daltons

Igs and CS in stool extracts and in saliva concentrated to more than 100 fold were studied by double diffusion using mono specific antisera (Nordic Hyland) IgA levels in urine saliva and stool extracts were measured by radio immuno assay technique (8 9)

Stool specimens with adequate IgA levels were studied by immunoelectrophoresis using various anti SC antisera (Nordic and prepared by author (TT)) to see if the SC would migrate with the IgA indicating SIgA These specimens were also studied by an immunoselection technique in which anti *free* SC antibody (Behring) was incorporated in the gel and the specimen reacted in double diffusion against anti IgA and an anti SC antibody (Nordic) capable of reacting with SC *either* free or in association with IgA

Specimens obtained by biopsy and autopsy tissues were frozen in liquid nitrogen and stained within two months with fluoresceinated antisera (Hyland) (10)

Marrow cells were examined for intracellular Igs by immunofluorescence techniques by Dr J Vossen (18)

RESULTS

Our patient s serum Ig levels were normal or elevated during her first months of life but after nine months IgG and IgM levels became subnormal (Fig 1) Serum IgA levels remained normal This IgA of subclass A_1 was largely IgA κ and migrated primarily cathodally on electrophoresis An antiserum specific for the conformational dimeric structure of IgA failed to precipitate IgA in the serum (14) Cytoplasmic immunofluorescence on marrow obtained at age 19 months identified a small number of IgA positive cells (Table 1) pre

Table 1 *Number of Ig containing cells in bone marrow as determined by immunofluorescence*

	Immunoglobulin						
	γ	α	μ	δ	κ	λ	κ/λ ratio
Patient	4	21	1	0	19	4	
Normal	122[b] (34–252)	109 (47–260)	60 (45–117)	0 9 (0–21)			1 65 0 81–2 44

Number of Ig containing cells per 10³ immunocytes (lymphocytes or plasma cells)
[b] Geometric mean plus range

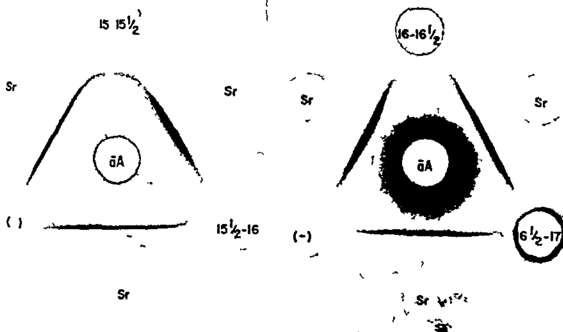


Fig 2 Double diffusion with anti IgA antisera (aA in center wells) Sr Serum diluted to a concentration of IgA of 10% mg (−) blank well Other wells contain saliva concentrated 40 times The numbers refer to the patient s age in months when the saliva was collected The decline in IgA levels with increasing age is evident

dominantly IgA κ type When marrow cells were stained with anti α chain antibody tagged with fluorescein isothiocyanate (FITC) in combination with anti κ chain antibody tagged with tetramethylrhodamine isothiocyanate (TRITC) 14α positive cells were found of which nine were also positive for κ When stained with anti-α chain FITC in combination with anti λ chain TRITC 16α positive cells were found none positive for λ

By double immunodiffusion IgA could be identified in saliva in trace amounts only when the saliva was concentrated at least 40 times and only before the 17th month of life (Figs 2 and 3) By radio immuno assay saliva IgA levels were less than 0 001 mg/dl (various samples) There was not enough material available to determine if this IgA was SIgA SC appeared quantitatively normal on double immunodiffusion Traces of IgG in saliva were also found during this period but IgM was not detected A lip biopsy in the 15th month showed no cells containing IgG IgM or IgA

IgA was easily detected in the patient s stools by double immunodiffusion in amounts grossly similar to those found in stools of five controls IgA levels by radio immuno assay were between 14 and 30 mg/dl in extracts of five patient stool samples whereas an age matched control stool sample contained 17 2 mg/dl (average of two measurements) This IgA was shown to be at least partly associated with SC by immunoelectrophoretic studies (Fig 3) and selective immunodiffusion IgM was not found in any of the patient s stools but they and two of five control stool samples contained traces of IgG

Radiologic study of the gut in the 15th month showed numerous small round filling defects jejunal and rectal biopsies showed these to represent lymphonodular aggregates Immunofluorescent staining of the specimens

IMMUNOELECTROPHORESIS
OF SALIVA AND STOOL EXTRACTS

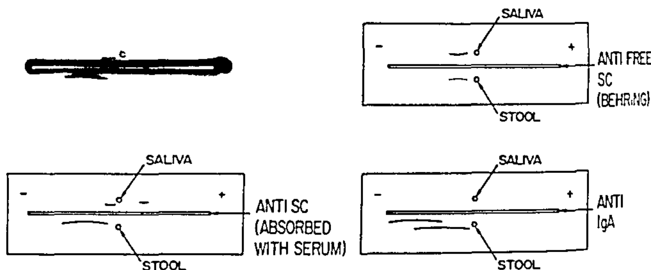


Fig. 3. Left side (photograph and diagram) shows the reaction of patient saliva and stool with an anti SC antiserum capable of reacting with SC *either* free or bound to IgA (anti SC (Nordic)). Similar analysis is shown diagrammatically on the right with an anti SC antiserum capable of reacting with *free* SC (anti SC (Behring)) (top) and with an anti IgA antiserum (bottom). Patient saliva shows an identical reaction with both SC antisera and no reaction with anti IgA indicating all the detectable SC is free. Stool extract shows a similar reaction with the anti IgA and the anti SC (Nordic) but only a trace reaction to the anti SC (Behring) indicating that most though not all of the detectable SC is associated with IgA, i.e. is SIgA. Additional precipitin lines seen in some instances may represent split products or cross reacting materials in enteric contents. Simultaneous studies done with substances containing known IgA, SIgA and SC confirmed their migration patterns under the conditions of the experiment.

showed no IgA containing cells but did reveal a few IgM and a very few IgG containing cells. At autopsy these lymphoid nodules were no longer found.

Radio immuno assay, capable of detecting as little as 0.015 mg/dl, showed no IgA in urine samples concentrated 30–40 times.

The numerous lymph nodes found at autopsy contained many pyroninophilic cells, plasma cells and a few germinal centers depleted of lymphocytes. Some of the plasma cells were fluorescent for IgG and IgM but not for IgA. Intestinal tissues at autopsy also showed no IgA containing cells.

DISCUSSION

Our patient had normal levels of monomeric serum IgA of restricted heterogeneity, largely IgA κ and of sub class A 1. Serum IgM and IgG decreased to subnormal levels during the early months of life. Many children with ADA negative SCID lack one or more classes of Ig; the reason for this is unclear. The decline of IgG and IgM is consistent with the immunologic regression often seen in SCID, particularly the ADA negative type. The only site where IgA containing cells were found was the marrow; it therefore seems likely that most, if not all, of the serum IgA was produced there.

Little or no IgA was found in urine or saliva. It is unlikely that salivary IgA was destroyed by proteolytic cleavage; this has not happened in saliva samples preserved frozen for several years by one of us (J. R.); furthermore lip biopsy showed no IgA containing cells. Therefore, despite normal levels of serum IgA, urinary and salivary IgA can be absent or extremely low.

During life and at autopsy samples of intestinal mucosa showed no IgA containing cells nevertheless in stool IgA was present at least partly bound to SC Our sampling may have missed the IgA containing cells though we consider this unlikely alternatively transudated serum IgA may have become attached to SC Finally dimeric IgA produced in the marrow may have been transported in plasma and secreted into the gut as occurs in normal individuals (12 13) Our failure to detect dimeric IgA in the serum does not negate this latter possibility as dimeric IgA in serum is normally present in very small amounts requiring special techniques for detection and may have been present at levels below the sensitivity of our tests

Our patient is the first with SCID in whom lymphonodular hyperplasia of the gut has been described This condition may be found in normal subjects but is most often seen with hypogammaglobulinemia or dysgammaglobulinemia The functional significance of this abnormality and its relation to B cell development the SIgA system and ADA remains unknown

Other patients have been described with partial deficiencies in their IgA systems Rubinstein et al described a boy with SCID who had normal IgA levels in serum jejunal secretions and stool but no detectable IgA in saliva urine and tears (15) Studies for SIgA were not done and his ADA status is unknown Strober et al described a 15 year-old boy with normal levels of serum IgA very low levels of IgA and undetectable SC in intestinal secretions and saliva his IgA abnormalities were thought secondary to the SC deficiency (16) Krakauer et al described an adult who died with renal failure and severe malabsorption who had normal serum IgA levels and markedly decreased SIgA in secretions (2)

Our current concepts of IgA SIgA and B cell development do not adequately explain these discrepancies between serum IgA and SIgA Unknown factors possibly related to ADA function appear to be capable of influencing the development of B cell immunity to produce compartmentalization of the IgA system

ACKNOWLEDGMENTS

We thank Drs J Vossen and G Scherer for immunofluorescent studies and I H Porter for critical review of the paper Ms D Schreinemackers and R Null gave technical and secretarial assistance respectively

REFERENCES

1 Giblett E R Anderson J E Cohen F Pollara B & Meuwissen H J Adenosine deaminase deficiency in two patients with severely impaired cellular immunity *Lancet II* 1067 1972

2 Krakauer R Zinneman H H & Hong R Deficiency of secretory IgA and intestinal malabsorption *Am J Gastroenterol 64* 319 1975

3 Mancini G Carbonara A O & Heremans J F Immunochemical quantitation of antigens by single radial immunodiffusion *Immunochemistry 2* 235 1965

4 Mestecky J & Lawton A Report of the International Symposium on the Immunoglobulin A System Birmingham Alabama 1973 In *Advances in experimental medicine and biology* Plenum Press New York and London 1974 vol 45 p 446

5 Meuwissen H J Pickering R J Pollara B & Porter I H *Combined immunodeficiency disease and adenosine deaminase deficiency A molecular defect* Academic Press New York 1975

6 Meuwissen H J Pollara B & Pickering R J Combined immunodeficiency disease associated with ADA deficiency *J Ped atr 86* 169 1975

7 Moore E C Pollara B Propp R & Meuwissen H J Combined immunodeficiency of the ADA deficient type with reconstitution of B cell function after maternal marrow graft To be published in the report of the Second Albany Conference on ADA Deficiency

8 Morgan C & Lazarow A Immunoassay of insulin using a two antibody system *Proc Soc Exp Biol Med 110* 29 1962

9 Morrison M Boyse G & Webster R Use of lactoperoxidase catalized iodinization in immunochemical studies *Immunochemistry 8* 89 1971

10 Ortega L G & Mellors R C The role of localized antibodies in the pathogenesis of nephrotic nephritis in the rat *J Exp Med 104* 151 1956

11 Radl J Light chain typing of immunoglobulins in small samples of biological material *Immunology 19* 137 1970

1 Radl J Schuit H R E Mestecky J & Hijmans W Report of the International Symposium on the Immunoglobulin A System Birmingham Alabama 1973 *In* J Mestecky & A Lawton (eds) *Advances in ex*

perimental medicine and biology Plenum Press New York and London 1974 vol 45 p 57
13 Radl J Swart A C W & Mestecky J The nature of the polymeric serum IgA in man *Proc Soc Exp Biol Med* 150 482 1975
14 Radl J Vandenberg P & Snoyink J J Antisera specific for human polymeric IgA *In* H Peeters (ed) *Protides of the biological fluids* Pergamon Press Oxford New York 1977
15 Rubinstein A Radl J Cottier H Rossie E & Gugler F Unusual combined immunodeficiency syndrome exhibiting Kappa IgD paraproteinemia residual gut immunity and graft versus host reaction after plasma infusion *Acta Paediatr Scand* 67 365 1973
16 Strober W Krakauer R Klaeveman H L Reynolds H Y & Nelson D L Secretory component deficiency A disorder of the IgA immune system *N Engl J Med* 294 351 1976
17 Swanson V Dyce B Citron P Rouleau C Feinstein D & Haverack B J Absence of IgA in serum with presence of IgA containing cells in intestinal tract *Clin Res* 16 119 1968 (Abstract)
18 Vossen J *The development of the B immune system in man* Thesis University of Leiden 1975

Submitted July 10 1978
Accepted Oct 7 1978

(H J M) Department of Pediatrics
Albany Medical College
Albany N Y 12208
USA

Acta Pædiatr Scand 68 459–464 1979

CASE REPORT

A VIRILIZING ADRENAL TUMOR WITH BORDERLINE ELEVATION OF URINARY 17 KETOSTEROIDS AND HISTOCHEMICAL DEMONSTRATION OF A DEFICIENCY IN THE Δ5/Δ4 ISOMERASE 3β HYDROXYSTEROID DEHYDROGENASE ENZYMATIC SYSTEM

R MONTELEONE NETO[1] J A MELLO DE OLIVEIRA[2] M F S SA[3] and J CORNICELLI

From the Departments of [1]Genetics [2]Pathology [3]Gynecology and Obstetrics and Surgery Faculty of Medicine of Ribeirao Preto University of Sao Paulo S P Brazil

ABSTRACT Monteleone Neto R Mello de Oliveira J A Sa M F S and Cornicelli J (Departments of Genetics Pathology Gynecology and Obstetrics and Surgery Faculty of Medicine Ribeirao Preto Brazil) A virilizing adrenal tumor with borderline elevation of urinary 17 ketosteroids and histochemical demonstration of a deficiency in the Δ5/Δ4 isomerase 3β hydroxysteroid dehydrogenase enzymatic system Acta Paediatr Scand 68 459 1979 —A 3-year-old girl affected by a virilizing tumor of the adrenal gland without significant elevation in the levels of 17 ketosteroids (17 KS) urinary excretion was studied clinically Her symptoms started abruptly at the age of 2 with progressive enlargement of the clitoris and the appearance of pubic hair In various tests the 17 KS levels barely exceeded the upper normal limits and at times remained within normal limits The retropneumoperitoneum X ray suggested an enlargement of the right adrenal gland and the presence of a neoplasm which was actually discovered during surgery Histopathological examination revealed a well defined neoplasm without capsule invasion and with accentuated cell polymorphism Histoenzymology showed that the tissue lacked the enzymatic system involving 3β hydroxysteroid dehydrogenase (3β HSD) Indoxylesterase (I EST A) activity identified the tumor as originating from the internal layers of the adrenal cortex The histochemical findings were correlated to the clinical picture and the levels of urinary 17 KS

KEY WORDS Virilizing adrenal tumor enzyme histochemistry 3β-hydroxysteroid dehydrogenase

Virilizing adrenal tumors frequently offer a characteristic clinical picture with a great increase in the urinary 17 KS levels and marked virilization Virilization associated with urinary 17 KS levels close to upper limit of normal is a rare occurrence (10 16 19) It has been suggested that in such cases an enzymatic blockage occurs in the tumor tissue due to the absence or reduced activity of one of the enzymes involved in steroidogenesis (2 5)

This paper describes a patient with an adrenal tumor which had virilizing effects but not accompanied by a marked elevation in urinary 17 KS An enzymatic defect in the synthesis of adrenocortical steroids was demonstrated

CASE REPORT

A 3 year-old white girl (R S P Reg 141778) was admitted to the hospital with a history of pubic hair growth and increased size of the clitoris for a period of one year During the same period of time the child s weight and height increased dramatically Her behaviour was aggressive and she was irritable but her general health was good On examination her height was 103 cm and weight 15 7 kg she had well developed subcutaneous and muscular tissue She had no acne or any axillary hair There was hair in the pubic and vulvar regions The labia majora were hypertrophic with skin rugosity the labia minora were hyperpigmented and hypertrophic The clitoris was peniform 5 cm long with hypertrophic cavernous bodies present (Fig 1) The urethral meatus was located in a

Research carried out under auspices of the Fundaçao de Amparo a Pesquisa do Estado de Sao Paulo—FAPESP —Grants Nos 70/1017 and 73/763

perimental medicine and biology Plenum Press New York and London 1974 vol 45 p 57
13 Radl J Swart A C W & Mestecky J The nature of the polymeric serum IgA in man *Proc Soc Exp Biol Med 150* 482 1975
14 Radl J Vandenberg P & Snoyink J J Antisera specific for human polymeric IgA *In* H Peeters (ed) *Protides of the biological fluids* Pergamon Press Oxford New York 1977
15 Rubinstein A Radl J Cottier H Rossie F & Gugler E Unusual combined immunodeficiency syndrome exhibiting Kappa IgD paraproteinemia residual gut immunity and graft versus host reaction after plasma infusion *Acta Paediatr Scand 62* 365 1973
16 Strober W Krakauer R Klaeveman H I Reynolds H Y & Nelson D I Secretory component deficiency A disorder of the IgA immune system *N Engl J Med 294* 351 1976
17 Swanson V Dyce B Citron P Rouleau C Feinstein D & Haverack B J Absence of IgA in serum with presence of IgA-containing cells in intestinal tract *Clin Res 16* 119 1968 (Abstract)
18 Vossen J *The development of the B immune system in man* Thesis University of Leiden 1975

Submitted July 10 1978
Accepted Oct 7 1978

(H J M) Department of Pediatrics
Albany Medical College
Albany N Y 12208
USA

CASE REPORT

A VIRILIZING ADRENAL TUMOR WITH BORDERLINE ELEVATION OF URINARY 17 KETOSTEROIDS AND HISTOCHEMICAL DEMONSTRATION OF A DEFICIENCY IN THE Δ5/Δ4 ISOMERASE 3β HYDROXYSTEROID DEHYDROGENASE ENZYMATIC SYSTEM

R MONTELEONE NETO[1] J A MELLO DE OLIVEIRA[2] M F S SA[3] and J CORNICELLI

From the Departments of Genetics [2]Pathology [3]Gynecology and Obstetrics and Surgery Faculty of Medicine of Ribeirao Preto University of Sao Paulo S P Brazil

ABSTRACT Monteleone Neto R Mello de Oliveira J A Sa M F S and Cornicelli J (Departments of Genetics Pathology Gynecology and Obstetrics and Surgery Faculty of Medicine Ribeirao Preto Brazil) A virilizing adrenal tumor with borderline elevation of urinary 17 ketosteroids and histochemical demonstration of a deficiency in the Δ5/Δ4-isomerase 3β hydroxysteroid dehydrogenase enzymatic system Acta Paediatr Scand 68 459 1979 —A 3 year-old girl affected by a virilizing tumor of the adrenal gland without significant elevation in the levels of 17 ketosteroids (17 KS) urinary excretion was studied clinically Her symptoms started abruptly at the age of 2 with progressive enlargement of the clitoris and the appearance of pubic hair In various tests the 17 KS levels barely exceeded the upper normal limits and at times remained within normal limits The retropneumoperitoneum X ray suggested an enlargement of the right adrenal gland and the presence of a neoplasm which was actually discovered during surgery Histopathological examination revealed a well defined neoplasm without capsule invasion and with accentuated cell polymorphism Histoenzymology showed that the tissue lacked the enzymatic system involving 3β hydroxysteroid dehydrogenase (3β HSD) Indoxylesterase (I EST A) activity identified the tumor as originating from the internal layers of the adrenal cortex The histochemical findings were correlated to the clinical picture and the levels of urinary 17 KS

KEY WORDS Virilizing adrenal tumor enzyme histochemistry 3β hydroxysteroid dehydrogenase

Virilizing adrenal tumors frequently offer a characteristic clinical picture with a great increase in the urinary 17 KS levels and marked virilization Virilization associated with urinary 17 KS levels close to upper limit of normal is a rare occurrence (10 16 19) It has been suggested that in such cases an enzymatic blockage occurs in the tumor tissue due to the absence or reduced activity of one of the enzymes involved in steroidogenesis (2 5)

This paper describes a patient with an adrenal tumor which had virilizing effects but not accompanied by a marked elevation in urinary 17 KS An enzymatic defect in the synthesis of adrenocortical steroids was demonstrated

CASE REPORT

A 3 year-old white girl (R S P Reg 141778) was admitted to the hospital with a history of pubic hair growth and increased size of the clitoris for a period of one year During the same period of time the child s weight and height increased dramatically Her behaviour was aggressive and she was irritable but her general health was good On examination her height was 103 cm and weight 15 7 kg she had well-developed subcutaneous and muscular tissue She had no acne or any axillary hair There was hair in the pubic and vulvar regions The labia majora were hypertrophic with skin rugosity the labia minora were hyperpigmented and hypertrophic The clitoris was peniform 5 cm long with hypertrophic cavernous bodies present (Fig 1) The urethral meatus was located in a

Research carried out under auspices of the Fundaçao de Amparo a Pesquisa do Estado de Sao Paulo—FAPESP —Grants Nos 70/1017 and 73/ 63



Fig 1 Hypertrophy of the clitoris and of the labia majora and minora with pubic hair growth in a female child affected by a virilizing neoplasm of the adrenal gland

low position near the hymenal orifice The vagina was 5 cm deep and the uterine cervix could be seen through a speculum No palpable tumor was found in the inguinal canal or in the labia majora The uterus could be felt on rectal examination

Serum levels of sodium potassium chloride glucose calcium cholesterol and blood urea were normal as well as all hematological and routine urinalysis The urinary gonadotrophin levels were less than 10 mouse units/24 h (3 determinations) The PBI was 7 4 μg/100 ml Plasma cortisol varied between 386 and 828 nmol/l (14 0–30 0 μg/100 ml) in 4 determinations The 17 ketogenic steroids varied between 9 3 and 24 2 μmol/24 h (2 7–7 0 mg/24 h) in 9 determinations Levels of 5 9 μmol/24 h (1 7 mg/24 h) for 17 hydroxysteroids and 2 2 μmol/24 h (0 7 mg/24 h) for pregnandiol were observed The 17 KS levels measured by the method of Drekter et al (3) fluctuated between 2 1 and 10 7 μmol/24 h (0 6–3 1 mg/24 h) in 9 determinations Sex chromatin was present and karyotype was 46 XX Bone age was evaluated as 6 years radiographs revealed a slight increase in size of the right adrenal gland

Laparotomy revealed a lobated tumor of the right adrenal gland deeply embedded in the normal glandular tissue with approximate diameters of 2×3×3 cm There was increased vascularization but no adhesion of the tumor to neighbouring organs could be detected The appearance and dimensions of the left adrenal gland were normal The uterus ovaries tubes and remaining organs in the abdominal cavity appeared normal and without any apparent metastases The tumor was removed together with the non neoplastic tissue of the right adrenal gland

MATERIAL AND METHODS

The specimen was immediately sectioned to permit an examination of the relationship between the tumor and the cortex and medulla of the adrenal The tumor roughly spherical in shape and well-defined was located within the cortical tissue It had an approximate diameter of 2 cm and was golden yellow in color differentiating it from the surrounding tissues A 3 mm thick section including both neoplastic and normal tissue was dipped into liquid nitrogen (−196 C) for 30 sec transferred to an isopor container filled with dry ice and carried to the laboratory for histochemical tests The remaining material was fixed in formalin (10%) and processed routinely for the histopathology examination

Sections 10 microns thick were obtained from the frozen fragment with a model Harris CTD cryotome (The International Equipment Company Needham Heights Mass) The sections were incubated in histochemical media for the detection of the following enzymatic activities primary alcohol dehydrogenase (A D I) secondary alcohol dehydrogenase (A D II) glucose-6-phosphate dehydrogenase (G6PD) 6-phosphogluconate dehydrogenase (6PG D) NADH$_2$ tetrazolium reductase (NADH$_2$ TR) NADPH$_2$ tetrazolium reductase (NADPH$_2$ TR) 3β hydroxysteroid-dehydrogenase (3β HSD) lactate dehydrogenase (L D) malate dehydrogenase (M D) succinate dehydrogenase (S D) tween-esterase (T EST A) indoxylesterase (I EST A) naphthol esterase (N EST A) alkaline phosphatase (GP AI) acid phosphatase (αGP A II) monoamine oxidase (MAO) and β glucuronidase (βGL A) Sections were also stained with periodic acid Schiff (PAS) toluidine blue Sudan Black and Brachet reaction for DNA and RNA Formalin fixed specimens were embedded in paraffin and stained with hematoxylin/eosin

RESULTS

Histopathological examination revealed a tumor of the adrenal cortex with intense cell polymorphism nuclear hyperchromatism and a few undifferentiated areas There was no invasion of the adrenal capsule or of the nearby blood and lymph vessels It was not possible to determine from the histopathology whether the neoplasm was malignant or not

The results of the histochemical examination are summarized in Table 1 where the activities of enzymes from normal and neoplastic glandular tissue are listed In the nor



Fig 2 Histochemical oxidation reduction reaction of dehydroepiandrosterone (*A*) in the non neoplastic cortex (1) the medulla (?) and the neoplasm (3) For a comparison the result of NADP oxidation reduction by the same tissues (*B*) performed in a section close to the previous one

mal cortical area the enzymes associated with the pentose phosphate pathway i e G6P D 6PG D and NAPDH$_3$ TR has strong activity Among the enzymes directly associated with the synthesis of cortical steroid hormones A DH had weak to moderate activity and 3β HSD moderate to strong activity both enzymes being present in every cortical layer In the innermost cortical layer 3β HSD usually of weak intensity showed a non uniform distribution with alternating areas of moderate and high activity (Fig 2) Table 1 shows also the enzymatic activities associated with energy metabolism and the metabolism of lipids and proteins According to Holt (12) the I EST A reaction is absent in the glomerular layer of the cortex with activity increasing gradually in the fascicular and reticular layers In this study N EST A was always weaker than I EST A and was distributed throughout all cortical layers with uniform intensity The enzymes associated with glycolysis Krebs cycle and pentose pathway showed weaker activity in the medullary section of the adrenal than in the cortical region The enzymes associated with steroidogenesis and lipid metabolism were absent in the medulla

All enzyme activities detected in the normal cortex were also present in the neoplastic tissue except for 3β HSD which showed practically no activity except in a few isolated neoplastic cells (Fig 2)

A sizable reduction in the activity of enzymes of pentose phosphate pathway can be observed in the neoplasm It is interesting to note that the neoplastic tissue shows identical I EST A activity to that of the deeper layers of the cortex MAO activity was more intense in the neoplastic tissue than in the other areas of glandular tissue A sizable increase in L D



Fig. 1 Hypertrophy of the clitoris and of the labia majora and minora with pubic hair growth in a female child affected by a virilizing neoplasm of the adrenal gland

low position near the hymenal orifice The vagina was 5 cm deep and the uterine cervix could be seen through a speculum No palpable tumor was found in the inguinal canal or in the labia majora The uterus could be felt on rectal examination

Serum levels of sodium potassium chloride glucose calcium cholesterol and blood urea were normal as well as all hematological and routine urinalysis The urinary gonadotrophin levels were less than 10 mouse units/24 h (3 determinations) The PBI was 7 4 μg/100 ml Plasma cortisol varied between 386 and 828 nmol/l (14 0–30 0 μg/100 ml) in 4 determinations The 17 ketogenic steroids varied between 9 3 and 24 2 μmol/24 h (2 7–7 0 mg/24 h) in 9 determinations Levels of 5 9 μmol/24 h (1 7 mg/24 h) for 17 hydroxysteroids and 2 2 μmol/24 h (0 7 mg/24 h) for pregnandiol were observed The 17 KS levels measured by the method of Drekter et al (3) fluctuated between 2 1 and 10 7 μmol/24 h (0 6–3 1 mg/24 h) in 9 determinations Sex chromatin was present and karyotype was 46 XX Bone age was evaluated as 6 years radiographs revealed a slight increase in size of the right adrenal gland

Laparotomy revealed a lobated tumor of the right adrenal gland deeply embedded in the normal glandular tissue with approximate diameters of 2×3×3 cm There was increased vascularization but no adhesion of the tumor to neighbouring organs could be detected The appearance and dimensions of the left adrenal gland were normal The uterus ovaries tubes and remaining organs in the abdominal cavity appeared normal and without any apparent metastases The tumor was removed together with the non neoplastic tissue of the right adrenal gland

MATERIAL AND METHODS

The specimen was immediately sectioned to permit an examination of the relationship between the tumor and the cortex and medulla of the adrenal The tumor roughly spherical in shape and well-defined was located within the cortical tissue It had an approximate diameter of 2 cm and was golden yellow in color differentiating it from the surrounding tissues A 3 mm thick section including both neoplastic and normal tissue was dipped into liquid nitrogen (−196 C) for 20 sec transferred to an isopor container filled with dry ice and carried to the laboratory for histochemical tests The remaining material was fixed in formalin (10%) and processed routinely for the histopathology examination

Sections 10 microns thick were obtained from the frozen fragment with a model Harris CTD cryotome (The International Equipment Company Needham Heights Mass) The sections were incubated in histochemical media for the detection of the following enzymatic activities primary alcohol dehydrogenase (A D I) secondary alcohol dehydrogenase (A DII) glucose-6-phosphate dehydrogenase (G6PD) 6-phosphogluconate dehydrogenase (6PG D) $NADH_2$ tetrazolium reductase ($NADH_2$ TR) $NADPH_2$ tetrazolium reductase (NADPH-TR) 3β hydroxysteroid-dehydrogenase (3β HSD) lactate dehydrogenase (L D) malate-dehydrogenase (M D) succinate dehydrogenase (S D) tween-esterase (T EST A) indoxylesterase (I EST A) naphthol-esterase (N EST A) alkaline phosphatase (GP AI) acid phosphatase (αGP A II) monoamine oxidase (MAO) and β glucuronidase (βGL A) Sections were also stained with periodic acid Schiff (PAS) toluidine blue Sudan Black and Brachet reaction for DNA and RNA Formalin fixed specimens were embedded in paraffin and stained with hematoxylin/eosin

RESULTS

Histopathological examination revealed a tumor of the adrenal cortex with intense cell polymorphism nuclear hyperchromatism and a few undifferentiated areas There was no invasion of the adrenal capsule or of the nearby blood and lymph vessels It was not possible to determine from the histopathology whether the neoplasm was malignant or not

The results of the histochemical examination are summarized in Table 1 where the activities of enzymes from normal and neoplastic glandular tissue are listed In the nor



Fig 2 Histochemical oxidation reduction reaction of dehydroepiandro terone (*A*) in the non neoplastic cortex (1) the medulla (2) and the neoplasm (3) For a com parison the result of NADP oxidation reduction by the same tissues (*B*) performed in a section close to the pre vious one

mal cortical area the enzymes associated with the pentose phosphate pathway i e G6P D 6PG D and NAPDH$_2$ TR has strong activity Among the enzymes directly associated with the synthesis of cortical steroid hormones A DH had weak to moderate activity and 3β HSD moderate to strong activity both enzymes being present in every cortical layer In the innermost cortical layer 3β HSD usually of weak intensity showed a non uniform distribution with alternating areas of moderate and high activity (Fig 2) Table 1 shows also the enzymatic activities associated with energy metabolism and the metabolism of lipids and proteins According to Holt (12) the I EST A reaction is absent in the glomerular layer of the cortex with activity increasing gradually in the fascicular and reticular layers In this study N EST A was always weaker than I EST A and was distributed throughout all cortical layers with uniform intensity The enzymes associated with glycolysis Krebs cycle and pentose pathway showed weaker activity in the medullary section of the adrenal than in the cortical region The enzymes asso ciated with steroidogenesis and lipid metab olism were absent in the medulla

All enzyme activities detected in the normal cortex were also present in the neoplastic tis sue except for 3β HSD which showed prac tically no activity except in a few isolated neo-plastic cells (Fig 2)

A sizable reduction in the activity of en zymes of pentose phosphate pathway can be observed in the neoplasm It is interesting to note that the neoplastic tissue shows identical I EST A activity to that of the deeper layers of the cortex MAO activity was more intense in the neoplastic tissue than in the other areas of glandular tissue A sizable increase in L D



Fig. 1 Hypertrophy of the clitoris and of the labia majora and minora with pubic hair growth in a female child affected by a virilizing neoplasm of the adrenal gland

low position near the hymenal orifice The vagina was 5 cm deep and the uterine cervix could be seen through a speculum No palpable tumor was found in the inguinal canal or in the labia majora The uterus could be felt on rectal examination

Serum levels of sodium potassium chloride glucose calcium cholesterol and blood urea were normal as well as all hematological and routine urinalysis The urinary gonadotrophin levels were less than 10 mouse units/24 h (3 determinations) The PBI was 7 4 μg/100 ml Plasma cortisol varied between 386 and 828 nmol/l (14 0–30 0 μg/100 ml) in 4 determinations The 17 ketogenic steroids varied between 9 3 and 24 2 μmol/24 h (2 7–7 0 mg/24 h) in 9 determinations Levels of 5 9 μmol/24 h (1 7 mg/24 h) for 17 hydroxysteroids and 2 2 μmol/24 h (0 7 mg/24 h) for pregnandiol were observed The 17 KS levels measured by the method of Drekter et al (3) fluctuated between 2 1 and 10 7 μmol/24 h (0 6–3 1 mg/24 h) in 9 determinations Sex chromatin was present and karyotype was 46 XX Bone age was evaluated as 6 years radiographs revealed a slight increase in size of the right adrenal gland

Laparotomy revealed a lobated tumor of the right adrenal gland deeply embedded in the normal glandular tissue with approximate diameters of 2×3×3 cm There was increased vascularization but no adhesion of the tumor to neighbouring organs could be detected The appearance and dimensions of the left adrenal gland were normal The uterus ovaries tubes and remaining organs in the abdominal cavity appeared normal and without any apparent metastases The tumor was removed together with the non neoplastic tissue of the right adrenal gland

MATERIAL AND METHODS

The specimen was immediately sectioned to permit an examination of the relationship between the tumor and the cortex and medulla of the adrenal The tumor roughly spherical in shape and well-defined was located within the cortical tissue It had an approximate diameter of 2 cm and was golden yellow in color differentiating it from the surrounding tissues A 3 mm thick section including both neoplastic and normal tissue was dipped into liquid nitrogen (−196 C) for 20 sec transferred to an isopor container filled with dry ice and carried to the laboratory for histochemical tests The remaining material was fixed in formalin (10%) and processed routinely for the histopathology examination

Sections 10 microns thick were obtained from the frozen fragment with a model Harris CTD cryotome (The International Equipment Company Needham Heights Mass) The sections were incubated in histochemical media for the detection of the following enzymatic activities primary alcohol dehydrogenase (A D I) secondary alcohol dehydrogenase (A D II) glucose-6-phosphate-dehydrogenase (G6PD) 6-phosphogluconate dehydrogenase (6PG D) NADH tetrazolium reductase ($NADH_2$ TR) $NADPH_2$ tetrazolium reductase (NADPH-TR) 3β hydroxysteroid-dehydrogenase (3β HSD) lactate dehydrogenase (L D) malate-dehydrogenase (M D) succinate dehydrogenase (S D) tween-esterase (T EST A) indoxylesterase (I EST A) naphthol esterase (N EST A) alkaline phosphatase (GP AI) acid phosphatase (αGP A II) monoamine oxidase (MAO) and β glucuronidase (βGL A) Sections were also stained with periodic acid Schiff (PAS) toluidine blue Sudan Black and Brachet reaction for DNA and RNA Formalin fixed specimens were embedded in paraffin and stained with hematoxylin/eosin

RESULTS

Histopathological examination revealed a tumor of the adrenal cortex with intense cell polymorphism nuclear hyperchromatism and a few undifferentiated areas There was no invasion of the adrenal capsule or of the nearby blood and lymph vessels It was not possible to determine from the histopathology whether the neoplasm was malignant or not

The results of the histochemical examination are summarized in Table 1 where the activities of enzymes from normal and neoplastic glandular tissue are listed In the nor



2 Histochemical oxidation reduction reaction of [d]roepiandrosterone (*A*) in the non neoplastic cortex [t]he medulla (?) and the neoplasm (3) For a com parison the result of NADP oxidation reduction by the same tissues (*B*) performed in a section close to the pre vious one

cortical area the enzymes associated with pentose phosphate pathway i e G6P D [6P]G D and NAPDH$_2$-TR has strong activity [Am]ong the enzymes directly associated with synthesis of cortical steroid hormones [D]1I had weak to moderate activity and [3β] HSD moderate to strong activity both [enz]ymes being present in every cortical layer the innermost cortical layer 3β HSD [usu]ally of weak intensity showed a non [uni]form distribution with alternating areas of [mo]derate and high activity (Fig 2) Table 1 [sh]ows also the enzymatic activities associated [wi]th energy metabolism and the metabolism of [lip]ids and proteins According to Holt (12) the [I] EST A reaction is absent in the glomerular [la]yer of the cortex with activity increasing [gr]adually in the fascicular and reticular layers [In] this study N EST A was always weaker [th]an I EST A and was distributed throughout all cortical layers with uniform intensity The enzymes associated with glycolysis Krebs cycle and pentose pathway showed weaker activity in the medullary section of the adrenal than in the cortical region The enzymes asso ciated with steroidogenesis and lipid metab olism were absent in the medulla

All enzyme activities detected in the normal cortex were also present in the neoplastic tis sue except for 3β HSD which showed prac tically no activity except in a few isolated neo plastic cells (Fig 2)

A sizable reduction in the activity of en zymes of pentose phosphate pathway can be observed in the neoplasm It is interesting to note that the neoplastic tissue shows identical I EST A activity to that of the deeper layers of the cortex MAO activity was more intense in the neoplastic tissue than in the other areas of glandular tissue A sizable increase in L D



Fig. 1 Hypertrophy of the clitoris and of the labia majora and minora with pubic hair growth in a female child affected by a virilizing neoplasm of the adrenal gland

low position near the hymenal orifice The vagina was 5 cm deep and the uterine cervix could be seen through a speculum No palpable tumor was found in the inguinal canal or in the labia majora The uterus could be felt on rectal examination

Serum levels of sodium potassium chloride glucose calcium cholesterol and blood urea were normal as well as all hematological and routine urinalysis The urinary gonadotrophin levels were less than 10 mouse units/24 h (3 determinations) The PBI was 7 4 μg/100 ml Plasma cortisol varied between 386 and 828 nmol/l (14 0–30 0 μg/100 ml) in 4 determinations The 17 ketogenic steroids varied between 9 3 and 24 2 μmol/24 h (2 7–7 0 mg/24 h) in 9 determinations Levels of 5 9 μmol/24 h (1 7 mg/24 h) for 17 hydroxysteroids and 2 2 μmol/24 h (0 7 mg/24 h) for pregnandiol were observed The 17 KS levels measured by the method of Drekter et al (3) fluctuated between 2 1 and 10 7 μmol/24 h (0 6–3 1 mg/24 h) in 9 determinations Sex chromatin was present and karyotype was 46 XX Bone age was evaluated as 6 years radiographs revealed a slight increase in size of the right adrenal gland

Laparotomy revealed a lobated tumor of the right adrenal gland deeply embedded in the normal glandular tissue with approximate diameters of 2×3×3 cm There was increased vascularization but no adhesion of the tumor to neighbouring organs could be detected The appearance and dimensions of the left adrenal gland were normal The uterus ovaries tubes and remaining organs in the abdominal cavity appeared normal and without any apparent metastases The tumor was removed together with the non neoplastic tissue of the right adrenal gland

MATERIAL AND METHODS

The specimen was immediately sectioned to permit an examination of the relationship between the tumor and the cortex and medulla of the adrenal The tumor roughly spherical in shape and well-defined was located within the cortical tissue It had an approximate diameter of 2 cm and was golden yellow in color differentiating it from the surrounding tissues A 3 mm thick section including both neoplastic and normal tissue was dipped into liquid nitrogen (−196 C) for 20 sec transferred to an isopor container filled with dry ice and carried to the laboratory for histochemical tests The remaining material was fixed in formalin (10%) and processed routinely for the histopathology examination

Sections 10 microns thick were obtained from the frozen fragment with a model Harris CTD cryotome (The International Equipment Company Needham Heights Mass) The sections were incubated in histochemical media for the detection of the following enzymatic activities primary alcohol dehydrogenase (A D I) secondary alcohol dehydrogenase (A DII) glucose-6-phosphate-dehydrogenase (G6PD) 6-phosphogluconate dehydrogenase (6PG D) NADH tetrazolium reductase ($NADH_2$ TR) $NADPH_2$ tetrazolium reductase ($NADPH_2$ TR) 3β hydroxysteroid-dehydrogenase (3β HSD) lactate dehydrogenase (L D) malate-dehydrogenase (M D) succinate dehydrogenase (S D) tween-esterase (T EST A) indoxylesterase (I EST A) naphthol-esterase (N EST A) alkaline phosphatase (GP AI) acid phosphatase (αGP AII) monoamine oxidase (MAO) and β glucuronidase (βGL A) Sections were also stained with periodic acid Schiff (PAS) toluidine blue Sudan Black and Brachet reaction for DNA and RNA Formalin fixed specimens were embedded in paraffin and stained with hematoxylin/eosin

RESULTS

Histopathological examination revealed a tumor of the adrenal cortex with intense cell polymorphism nuclear hyperchromatism and a few undifferentiated areas There was no invasion of the adrenal capsule or of the nearby blood and lymph vessels It was not possible to determine from the histopathology whether the neoplasm was malignant or not

The results of the histochemical examination are summarized in Table 1 where the activities of enzymes from normal and neoplastic glandular tissue are listed In the nor

of 3β HSD when dehydroepiandrosterone is utilized as a substrate is weaker in the reticular zone thus confirming the results of Goldman et al (5) for children of this age In our case however the reaction intensity in this layer is not uniform showing focal points of activity very close to that of the external layers The validity of a histochemical technique for the demonstration of 3β HSD activity in human adrenal cortex was also studied by Goldman et al (5) who showed the parallelism between results obtained by biochemical and histochemical techniques The tumor that developed in the cortex did not show the cortical type of activity in the overwhelming majority of its cell population Consequently this neoplasm acted as an independent gland incapable of synthetizing adequate adrenocortical steroids and carrying out an anomalous biosynthetic pathway characterized by Δ5 compounds In such a biosynthetic pathway there are androgenic compounds capable of producing some virilization One such instance occurs in the congenital adrenal Bongiovanni type hyperplasia in which there is a lack of activity of 3β HSD with virilization of external female genitalia and urinary excretion of Δ5 3β ol compounds (1) but with high levels of urinary 17 KS suggesting that in tumor tissues another kind of hormone may possibly have been produced or another enzyme could be deficient too

The advanced bone age of the present case could indicate increased DHA production This possibility however could not be confirmed since no measurements were made of blood or urinary levels of DHA and other compounds of the steroidogenic pathway The nearby non neoplastic tissue which showed a positive reaction for 3β HSD activity also acted as a control for the reaction of the tumor tissue This demonstrates that the latter was unable to carry out C_3 oxidation in the cyclopentanophenanthrene ring thus confirming the hypothesis advanced by David et al (2) Goldman et al (5) demonstrated this same blockage in cases of hyperplasias adenomas and carcinomas of the adrenal gland in which the high excretion of Δ5 3β HSD products gave rise to the hypothesis of a decrease in the activity of 3β HSD which was then confirmed both histochemically and biochemically In agreement Hoch Ligeti (11) always found a positive reaction for 3β HSD in non virilizing adrenal adenomas

The oxidation reduction of C_3 is carried out by an enzymatic system involving actual C_3 oxidation reduction and isomerization of cyclopentanophenanthrene nucleus from its Δ5 to its Δ4 form in which a specific isomerase takes part The histochemical method applied utilizes DHA as a substrate which is a steroid compound with a hydroxyl group in C_3 position and a double bond between C_5 and C_6 i e a 3β hydroxy Δ5 compound Thus the final result of the reaction demonstrates the joint action of oxidoreductase and isomerase The absence of formazan the final product of nitro blue tetrazolium reduction and the indicator of reaction intensity in the neoplastic tissue does not permit us to distinguish between the lack of one or the other of the enzymes involved

In the present case we found that the I EST A reaction was positive in the non neoplastic cortical cells starting from the fascicular zone of the cortex and increasing in activity towards the reticular zone The presence of the same enzymatic activity in the neoplastic tissue shows a differentiation in function for the tumor cells which is identical to that for the internal layers of the adrenal cortex and suggests that this neoplasm originates in the innermost cells of the cortical layer I EST A activity is interpreted as an enzymatic activity connected with lipid metabolism The present study shows that this activity involves enzymes other than the esterases which hydrolyze Tween 80 (T EST A) and naphthol acetate (N EST A) since the results of the three reactions are completely independent from the point of view of both intensity and topography

The histochemical data allows us to con

Table 1. *Histoenzymology of a virilizing neoplasm of the adrenal cortex and of the neighbouring non neoplastic tissue*

Enzymatic activity scale: n=negative; +=weak reaction; ++=moderate reaction; +++=strong reaction; ++++=very intense reaction; tumor=neoplastic tissue; Glom.=glomerular; Fascic.=fascicular; Retic.=reticular; M.P.S.=mucopolysaccharides; Med.=medulla. For further details see text.

Metabolic pathway	Reaction	Tumor	Normal cortex Glom.	Fascic.	Retic.	Med.	Technique
Pentose pathway	G6PD	++	++++	+++	+++	+	Wegman et al. (21)
Pentose pathway	6PGD	++	++	+	+	n	Wegman et al. (21)
Pentose pathway	NADPH$_2$-TR	++++	++++	+++	++++	+	Scarpelli et al. (17)
Steroidogenesis	ADH	++	++	+	+	n	Hardonk (9)
Steroidogenesis	3βHSD	n	+++	++	++	n	Levy et al. (13)
Glycolysis	ALD	+	++	+	+	n	Hardonk (9)
Glycolysis	LD	+++	++	++	++	++	Nachlas et al. (15)
Krebs cycle	MD	++	+++	++	++	++	Nachlas et al. (15)
Krebs cycle	SD	+/++	+	+	+	+	Wegman et al. (20)
Krebs cycle	NADH$_2$-TR	++++	++++	+++	+++	++	Scarpelli et al. (17)
Lipid metabolism	TEST A	n	n	n	n	n	Gomori (8)
Lipid metabolism	TEST A	+++	n	++	+++	n	Holt (12)
Lipid metabolism	N EST A	+	+	+	+	n	Nachlas et al. (14)
Lipid metabolism	αGPA I	n	n	n	n	n	Gomori (6)
Lipid metabolism	αGPA II	+	+	+	+	+	Gomori (7)
Amino acid metabolism	MAO	++	+	+	+	+	Glenner et al. (4)
M.P.S. metabolism	βGLA	n	n	n	n	n	Seligman et al. (18)

In this reaction there are a few isolated cells showing enzymatic activity.

activity was also found in the neoplasm and enzymes that are inactive in normal cortex were also inactive in the neoplasm.

DISCUSSION

A few cases of female patients affected by virilizing tumors of the adrenal gland not associated with elevated levels of urinary 17 KS have been described in the literature (10, 16, 19). This type of unusual association makes clinical diagnosis more difficult. In the usual cases associated with high levels of urinary 17 KS, the adrenal suppression test confirms the diagnosis, since after the administration of suppressive drugs there is no reduction of its excretory levels (23).

Some clinical signs might be present which will help make the diagnosis and indicate the necessity for surgical treatment. These are: post natal onset of progressive and rapid virilization, exaggerated growth of the clitoris and accelerated bone maturation. All of these signs were present in this patient, who started showing clitoral hypertrophy at 2 years of age and had a bone age of approximately 6 years at a chronological age of 3 years.

The lack of increase in the levels of urinary 17 KS in such cases has been the object of various speculations, one of which is the hypothesis advanced by David et al. (2). They suggest that both the low 17 KS levels and the elevation in blood dehydroepiandrosterone (DHA) sulphate described in these cases could be explained by an enzymatic error in the tumor tissue. This enzymatic error would divert normal steroidogenesis towards the biosynthesis of androgenic non keto hormones or supply a different kind of steroid that are converted in peripheral tissues to mere potent androgenic non keto hormones. According to David et al. (2), 3β HSD could be the deficient enzyme.

In our study we assessed the activity of various enzymes, including 3β HSD, by histochemical techniques. The normal cortex has strong activity in every layer, intensity being highest in the glomerular tissue. The activity

of 3β HSD when dehydroepiandrosterone is utilized as a substrate is weaker in the reticular zone thus confirming the results of Goldman et al (5) for children of this age In our case however the reaction intensity in this layer is not uniform showing focal points of activity very close to that of the external layers The validity of a histochemical technique for the demonstration of 3β HSD activity in human adrenal cortex was also studied by Goldman et al (5) who showed the parallelism between results obtained by biochemical and histochemical techniques The tumor that developed in the cortex did not show the cortical type of activity in the overwhelming majority of its cell population Consequently this neoplasm acted as an independent gland incapable of synthetizing adequate adrenocortical steroids and carrying out an anomalous biosynthetic pathway characterized by Δ5 compounds In such a biosynthetic pathway there are androgenic compounds capable of producing some virilization One such instance occurs in the congenital adrenal Bongiovanni type hyperplasia in which there is a lack of activity of 3β HSD with virilization of external female genitalia and urinary excretion of Δ5 3β ol compounds (1) but with high levels of urinary 17 KS suggesting that in tumor tissues another kind of hormone may possibly have been produced or another enzyme could be deficient too

The advanced bone age of the present case could indicate increased DHA production This possibility however could not be confirmed since no measurements were made of blood or urinary levels of DHA and other compounds of the steroidogenic pathway The nearby non neoplastic tissue which showed a positive reaction for 3β HSD activity also acted as a control for the reaction of the tumor tissue This demonstrates that the latter was unable to carry out C_3 oxidation in the cyclopentanophenanthrene ring thus confirming the hypothesis advanced by David et al (2) Goldman et al (5) demonstrated this same blockage in cases of hyperplasias adenomas and carcinomas of the adrenal gland in which the high excretion of Δ5 3β HSD products gave rise to the hypothesis of a decrease in the activity of 3β HSD which was then confirmed both histochemically and biochemically In agreement Hoch Ligeti (11) always found a positive reaction for 3β HSD in non virilizing adrenal adenomas

The oxidation reduction of C_3 is carried out by an enzymatic system involving actual C_3 oxidation reduction and isomerization of cyclopentanophenanthrene nucleus from its Δ5 to its Δ4 form in which a specific isomerase takes part The histochemical method applied utilizes DHA as a substrate which is a steroid compound with a hydroxyl group in C_3 position and a double bond between C_5 and C_6 i e a 3β hydroxy Δ5 compound Thus the final result of the reaction demonstrates the joint action of oxidoreductase and isomerase The absence of formazan the final product of nitro blue tetrazolium reduction and the indicator of reaction intensity in the neoplastic tissue does not permit us to distinguish between the lack of one or the other of the enzymes involved

In the present case we found that the T EST A reaction was positive in the non neoplastic cortical cells starting from the fascicular zone of the cortex and increasing in activity towards the reticular zone The presence of the same enzymatic activity in the neoplastic tissue shows a differentiation in function for the tumor cells which is identical to that for the internal layers of the adrenal cortex and suggests that this neoplasm originates in the innermost cells of the cortical layer T EST A activity is interpreted as an enzymatic activity connected with lipid metabolism The present study shows that this activity involves enzymes other than the esterases which hydrolyze Tween 80 (T EST A) and naphthol acetate (N EST A) since the results of the three reactions are completely independent from the point of view of both intensity and topography

The histochemical data allows us to con

Table I *Histoenzymology of a virilizing neoplasm of the adrenal cortex and of the neighbouring non neoplastic tissue*

Enzymatic activity scale n=negative + =weak reaction ++=moderate reaction +++=strong reaction ++++ very intense reaction tumor=neoplastic tissue Glom =glomerular Fascic =fascicular Retic =reticular MPS=mucopolysaccharides Med =medulla For further details see text

Metabolic pathway	Reaction	Tumor	Normal cortex Glom	Fascic	Retic	Med	Technique
Pentose pathway	G6PD	++	++++	+++	+++	+	Wegman et al (21)
Pentose pathway	6PGD	++	++	+	+	n	Wegman et al (21)
Pentose pathway	$NADPH_2$ TR	++++	++++	+++	++++	+	Scarpelli et al (17)
Steroidogenesis	ADH	++	++	+	+	n	Hardonk (9)
Steroidogenesis	3βHSD	n	+++	++	++	n	Levy et al (13)
Glycolysis	ADI	+	++	+	+	n	Hardonk (9)
Glycolysis	LD	+++	++	++	++	++	Nachlas et al (15)
Krebs cycle	MD	++	+++	++	++	++	Nachlas et al (15)
Krebs cycle	SD	+/++	+	+	+	+	Wegman et al (20)
Krebs cycle	$NADH_2$ TR	++++	++++	+++	+++	++	Scarpelli et al (17)
Lipid metabolism	TEST A	n	n	n	n	n	Gomon (8)
Lipid metabolism	I EST A	+++	n	++	+++	n	Holt (12)
Lipid metabolism	N EST A	+	+	+	+	n	Nachlas et al (14)
Lipid metabolism	αGPA I	n	n	n	n	n	Gomon (6)
Lipid metabolism	αGPA II	+	+	+	+	+	Gomon (7)
Amino acid metabolism	MAO	++	+	+	+	+	Glenner et al (4)
MPS metabolism	βGLA	n	n	n	n	n	Seligman et al (18)

In this reaction there are a few isolated cells showing enzymatic activity

activity was also found in the neoplasm and enzymes that are inactive in normal cortex were also inactive in the neoplasm

DISCUSSION

A few cases of female patients affected by virilizing tumors of the adrenal gland not associated with elevated levels of urinary 17 KS have been described in the literature (10 16 19) This type of unusual association makes clinical diagnosis more difficult In the usual cases associated with high levels of urinary 17 KS the adrenal suppression test confirms the diagnosis since after the administration of suppressive drugs there is no reduction of its excretory levels (23)

Some clinical signs might be present which will help make the diagnosis and indicate the necessity for surgical treatment These are post natal onset of progressive and rapid virilization exaggerated growth of the clitoris and accelerated bone maturation All of these signs were present in this patient who started showing clitoral hypertrophy at 2 years of age and had a bone age of approximately 6 years at a chronological age of 3 years

The lack of increase in the levels of urinary 17 KS in such cases has been the object of various speculations one of which is the hypothesis advanced by David et al (2) They suggest that both the low 17 KS levels and the elevation in blood dehydroepiandrosterone (DHA) sulphate described in these cases could be explained by an enzymatic error in the tumor tissue This enzymatic error would divert normal steroidogenesis towards the biosynthesis of androgenic non keto hormones or supply a different kind of steroid that are converted in peripheral tissues to mere potent androgenic non keto hormones According to David et al (2) 3β HSD could be the deficient enzyme

In our study we assessed the activity of various enzymes including 3β HSD by histochemical techniques The normal cortex has strong activity in every layer intensity being highest in the glomerular tissue The activity

BOOK REVIEWS

D B Jelliffe & E F P Jelliffe *Human milk in the modern world* Oxford University Press 1978 500 pp illus £15 00 ISBN 0-19 64919 1

During the last few years breast feeding has become very much in the focus of interest Every major meeting of *paediatricians or nutrition* scientists has breast feeding and breast milk on the agenda Although breast milk is as old as mankind new constituents are still being discovered such as the water soluble Vitamin D and the many anti infective factors At present more than 100 components of breast milk are known

The newly awakened interest has been further enhanced by the recent greatly publicized court case brought about by Nestle against the action group Arbeitsgruppe der dritten Welt for distributing a pamphlet entitled Nestle kills babies This case has emphasized the extreme importance of prolonged breast feeding in poor and insanitary conditions particularly in developing countries However the subject of breast feeding in young child nutrition as part of the wider field of human food science has but a very modest share in the curricula of medical and nursing training This has been made very clear by the WHO Collaborative Breast Feeding Study soon to be published which deals partly with the training of health personnel Part of the problem and indicating the low priority of the subject until recently is the paucity of manuals and handbooks on breast feeding and breast milk An encouraging example has been set by the Swedish Board of Health and Welfare which has edited a factual unbiased manual on breast feeding and breast milk for health workers

Breast feeding is firmly associated with Derrick and Patrice Jelliffe and vice versa Over the years this industrious couple has written numerous scientific and popular articles on different aspects of the subject Among other things they have been very actively engaged in the fight against unethical marketing methods of the multinational baby food companies

The Jelliffes have now collected their considerable knowledge in an impressive volume of 500 pages entitled Human Milk in the Modern World Psychosocial nutritional and economic significance They have intended it for health professionals nutritionists community workers and national planners as well as for mothers groups

The book is based on a very extensive survey of the literature—the bibliography comprises close to 1 500 references and an additional last minute bibliography another 300 Seemingly every aspect of the subject is elucidated and in considerable detail including an interesting introductory chapter on Mammalian antecedents and adaptive suckling There we learn for instance that the blue whale has breast milk containing 50% fat to ensure a rapidly built up insulating fat layer in the young Also that the kangaroo is bean sized at birth (50 mg) but becomes firmly attached to the nipple in the pouch until mature its breast milk becomes less concentrated as the embryo grows and does not contain lactose If the young kangaroo is brought up on cow s milk it develops cataract just like the human baby with galactosaemia!

A large number of appropriate introductory quotations ranging from the Koran to modern thinkers and nursery rhymes make fascinating additions to the otherwise highly interesting chapters *The biological and biochemical* aspects are very proficiently elucidated from all angles and with all available up-to-date knowledge The Mother/Infant Dyad and particularly the importance of not separating the mother and child at artificial maternities is rightly pointed out Breast feeding will benefit greatly both in the short and long run from initiation of suckling within a few minutes or hours The worldwide economics of breast feeding including the consequences of early weaning are dealt with in great detail and interesting reviews are made of trends and influences in technological urban societies as well as in non western countries (Incidentally the term industrialized would seem more correct than Western" in these connections)

The Codes of Ethics recently published by the Ross Laboratories and by the ICIFI (ICIFI=International Council of Infant Food Industries) are given in the appendices with rather sarcastic criticism but no alternatives are offered or discussed

In the chapter on Practical programmes only one page is devoted to Formula for use when medically indicated for mothers unable or unwilling to breast feed No recipes or formulas are given for the benefit of the health worker who *has* to advise the mother in severe ill health or mothers working out of the home in paid employment where Westernized culture attitudes and facilities do not permit nursing The pendulum has swung from no-breast feeding to no-formula text books The need for a realistic balance should in my opinion be given greater emphasis

In 500 pages repetition and overlapping will inevitably occur and this book is no exception But page references are abundant for the benefit of the one-chapter reader

Human Milk in the Modern World is a major and commendable achievement although at times somewhat biased and unrealistic in its non acceptance of alternatives even when and where other forms of feeding (say between 3 and 6 months) are necessary for psychological or biological reasons or for reasons of social obligation It will most certainly become a classic and should be read by all those for whom it is intended

Yngve Hofvander

P B Beeson and D A Bass *The eosinophil* Holt Saunders Ltd Eastbourne 1978 £10 50 ISBN 0-7216-1650-X

Ever since Paul Ehrlich eighty years ago noticed the presence of a white blood cell with a peculiar affinity

clude that it is an adrenocortical functioning neoplasm originating in the internal layers of the adrenal cortex and bearing an enzymatic steroidogenesis defect which favors the formation of androgenic steroids Even though such steroids might be related to the associated virilizing symptoms it cannot be excluded on the basis of the results obtained that the hormones liberated by the tumor may have undergone further chemical transformations outside the adrenal gland (e g in the liver) leading to more powerful virilizing hormones

REFERENCES

1 Bongiovanni A M Adrenogenital syndrome with deficiency of 3βhydroxysteroid dehydrogenase *J Clin Invest 41* 2086 1962
2 David R Aufrest G & Gandey H M A virilizing adrenal tumor with borderline elevation of urinary 17 ketosteroids *Pediatrics 42* 139 1968
3 Drekter I J Heisler A Scism G R Stern S Person S & McGavack T H Determination of urinary steroids I Preparation of pigment free extracts and simplified procedure for estimation of total 17 ketosteroids *J Clin Endocrinol 12* 55 1952
4 Glenner G G Burtner H G & Brown G W The histochemical demonstration of monoamine oxidase activity by tetrazolium salts *J Histochem Cytochem 55* 591 1957
5 Goldman A S Bongiovanni A M Yakowac W C & Prader A Study of Δ5 3βhydroxysteroid dehydrogenase in normal hyperplastic and neoplastic adrenal cortical tissue *J Clin Endocrinol 24* 894 1964
6 Gomori G The distribution of phosphatase in normal organs and tissues *J Cell Comp Physiol 17* 71 1941
7 Gomori G Distribution of acid phosphatase in the tissue under normal and pathological conditions *Arch Pathol 32* 189 1942
8 Gomori G *Microscopic histochemistry* University of Chicago Press Chicago Ill 1952
9 Hardonk M J A new method for the histochemical demonstration of steroid producing cells in human tissues *Histochem 5* 234 1965
10 Hayles A B Hahn H B Jr Sprague R G Bahn R C & Priestley J T Hormone secreting tumors of the adrenal cortex in children *Pediatrics 37* 19 1966
11 Hoch Ligeti C Δ5 3β hydroxysteroid dehydrogenase activity in human adrenal cortical adenomas *Arch Pathol 93* 467 1972
12 Holt S J The value of fundamental studies of staining reactions in enzyme histochemistry with reference to indoxyl methods for esterase *J Histochem Cytochem 4* 541 1956
13 Levy H Deane H W & Rubin B L Visualization of steroid 3β OH dehydrogenase activity in tissues of intact and hypophysectomized rats *Endocrinology 65* 932 1959
14 Nachlas M M & Seligman A M The histochemical demonstration of esterase *J Nat Can Inst 9* 415 1949
15 Nachlas M M Walker D G & Seligman A M A histochemical method for the demonstration of diphosphopyridine nucleotide diaphorase *J Biophysic Biochem Cytol 4* 29 1958
16 Saez J M & Migeon C J Studies of androgen in patients with adrenocortical tumors *J Clin Endocrinol 27* 617 1967
17 Scarpelli D G Hess R & Pearse A G E The cytochemical localization of oxidative enzymes I Diphosphopyridine nucleotide diaphorase and triphosphopyridine nucleotide diaphorase *J Biophysic Biochem Cytol 4* 747 1958
18 Seligman A M Tsou K E Rutenberg S H & Cohen R B Histochemical demonstration of β D-glucuronidase with a synthetic substrate *J Histochem Cytochem 2* 209 1954
19 Toledo S P A Coelho-Neto A S Assis L M & Ulhoa Cintra A B Tumor virilizante de supra renal com elevaçao limitrofe dos niveis de 17-cetaesteroides urinários *Rev Ass Med Bras 19* 71 1973
20 Wegmann R & Tordet Candroit C Histochemical demonstration of succinic dehydrogenase by specific inhibition and activation *J Histochem Cytochem 8* 348 1960
21 Wegmann R & Gerzeli G La glucose 6-phosphate dehydrogenase et ses corrélations avec des substrats voisins du glucose 6 phosphate Role de l'hexokinase *Ann Histochim 6* 111 1961
22 Wegmann R & Sotelo C Aspects cytoenzymologiques du metabolisme des glucides de la cellule de Purkinje *Ann Histochim 7H* 65 1962
23 Wilkins L *Diagnostico y tratamiento de las enfermidades endocrinas en la infancia y la adolescencia* Espaxs Barcelona 1966

Submitted Sept 7 1977
Accepted Oct 17 1978

(R M N) Department of Medical Genetics
Faculty of Medicine
University of British Columbia
2075 Westbrook Place
Vancouver B C
Canada V6T 1W5

this they are well known for creativity and good results In one part of the book the situation of the nurses is described

There are two appendices and the first one Scenes in Ward 7 could very well be a first independent chapter Here Maureen Oswin describes everyday situations from the different wards in a dramatic and touching way Here you find things that the evening press could easily distort Maureen Oswin s description is objective and although the contents of the book as a whole is critical every chapter ends with constructive recommendations

There is a list of recommended further reading It is good but publications of James and Joyce Robertson are missing

The book is good reading for everybody who has to do with mentally handicapped children (medical consultants psychologists teachers nurses administrators etc) The book can be highly recommended and should be available in every hospital library

Per Vegfo s

E L Kendig Jr & V Chernick (eds) *Disorders of the spiratory tract in children* 3rd ed W B Saunders Company London 1115 pp illus £37 50 ISBN 0 7 16 5378 ?

With Dr Victor Chernick as a new associate editor and twenty two new contributors the third edition of Edwin L Kendig s Disorders of the respiratory tract in children has become a still better textbook in paediatric pneumology than the former editions All chapters have been r written or been revised and brought up to date The seven new chapters dealing with important paediatric disorders have made this edition a very comprehensive book covering almost all paediatri diseases of the lungs Most illustrations including roentgenograms are excellent and all chapters are followed by a lot of up-to date references Except some short chapters about disorders in adenoids and tonsils this edition is limited to disorders in the lower respiratory tract In spite of that it has become a large book containing sixty-eight chapters and almost 1100 pages It should be able to give answers to almost e ery clinical question about pulmonary diseases raised by physicians dealing with respiratory disorders in children

Hans Ahlstr m

J V Simone (Guest editor) *Acute leukaemia In* Clinics in Haematology June 1978 00 pp illus tabl W B Saunders Company Ltd $16 50

This book deals in particular with the therapeutic aspects of acute leukaemia Clinical features cytology and infectious complications are not covered in this issue of Clinics in haematology

J V Simone has an excellent foreword with short comments on the topics new since the 197? edition on acute leukaemia It is also interesting to note his remarks on editorial selection bias

Five of the ten chapters are of particular interest to paediatricians Stephen L George points out the problems of design and evaluation of leukaemia trials A Mauer presents the principles of treatment of acute leukaemia in children and the current protocol studies from St Jude Children s Hospital The heterogeneity of the high risk group is stressed The important discovery of abnormal computerized tomography of the brains of children receiving CNS-prophylaxis is mentioned and proposals for alternative CNS-prophylaxis not including irradiation are briefly commented on All cases of AML off therapy at St Jude Children s Hospital have subsequently relapsed

Saunders and Donald Thomas from the Seattle group present an excellent survey and discussion of bone marrow transplantation a procedure characterized by Simone as an experimental treatment method bone marrow transplantation has so far been most disappointing in leukaemias in Europe

CNS and gonadal involvement are dealt with by Aur and Hustu in a chapter on extramedullary leukaemia (sanctuaries) The well known first 7 studies from St Jude Children s Hospital (196 –1971) are presented particularly with respe t to their preventive CNS therapy The frequency of CNS relapse was 8/45 (17 %) in cases receiving cranial irradiation + methotrexate i t in study VII and the median duration of haematological remission 24 months (?3 months for craniospinal group)

The increasing incidence of testicular involvement is also mentioned (28 to 9? % at autopsy) Prophylactic irradiation of the testes is not used at St Jude Children s Hospital and only one testis is irradiated in cases on unilateral leukaemic involvement

Peter Johan Moe

for the acid dye eosin the eosinophil leukocyte has constantly puzzled its investigators. After reading the book by Beeson and Bass it is still far from clear what eosinophils do. The authors summarize not only in a purely descriptive but also in a highly critical way the existing knowledge of the basic nature of the eosinophil, its participation in phenomena of immunity, hormonal influences as well as extensive clinical observations of relevance to the subject.

At least the kinetics of the eosinophil seem well established today. It is likely that a key to the mysteries of what eosinophils do may lie in the specific functions of their granular proteins recently attracting increasing attention. The suggestion that the eosinophil has a homeostatic role in the immediate hypersensitivity reaction is of course highly likely: once in the inflammatory site it may interact with basophils and mast cells by several mechanisms. Otherwise a key factor in the genesis of eosinophilia appears to be processing of foreign material, e.g. parasites by cells in tissues. Interestingly, T lymphocytes seem necessary participants in the subsequently accelerated marrow production of eosinophils. T lymphocytes may produce colony stimulating factors specifically for eosinophilopoiesis. Also, a low molecular weight eosinophilopoetin candidate was recently detected in serum of eosinophil depleted animals. The characteristic effects of corticosteroids are most likely due to reversible sequestration of the cells within the vascular compartment or the spleen. The benefit of the eosinophil is to be expected but, as in the case of the neutrophil, it may produce harmful effects too. Indisputable evidence indicates that prolonged high blood levels of eosinophils can cause a specific form of heart disease with endocardial fibrosis.

The monograph by Beeson and Bass on the eosinophil provides a most useful book for laboratory investigators and physicians. It gives a good theoretical understanding and also shows our present lack of knowledge of the function of this cell.

Inge Olsson

P. L. Morselli (ed.) *Drug disposition during development.* 490 pp., illus. John Wiley & Sons Ltd., Sussex 1978. £28.20, $50.00. ISBN 0 89335 006 0.

It was not possible until some 10–15 years ago to study in detail the kinetics of drugs in children. The recent progress is a result of the development of sensitive analytical techniques, e.g. mass spectrometry, high pressure liquid chromatography etc. Most of the knowledge about drug disposition in children of various ages derives from the intense research during the last decade. The book by Paolo Morselli is an excellent and comprehensive monograph which brings together the current knowledge of drug disposition in the newborn, the infant and the child. Several authors, primarily from the "Mario Negri Institute" in Milan, where important investigations in this research area have been carried out, contribute to the fifteen chapters of the monograph. The book starts with a rather detailed chapter on basic concepts of pharmacokinetics. This may serve as a useful repetition for the reader. The next few chapters concern some general aspects of clinical pharmacology, namely: Drug Absorption, Drug Plasma Protein Binding and Distribution, Development of Drug Metabolizing Enzymes and Kidney Development, Drug Elimination Mechanisms with particular emphasis on the paediatric aspects. The other chapters describe the disposition of drugs belonging to certain groups: antineoplastic agents, antibiotics, chemotherapeutics, anticoagulants, antiinflammatory drugs, antiepileptics, hypnotics, cardiovascular agents and psychotropic drugs. All chapters are well written and seem to give a complete review of the current knowledge but would have gained a lot if the kinetic data had been tabulated to a larger extent. Also, there is no comparative evaluation of the drugs in each group. Nevertheless, the book can be used with ease as an encyclopedia. It should serve as a very useful reference for investigators and paediatricians with special interests in pharmacokinetics and therapeutics, for clinical pharmacologists, general practitioners and other medical people interested in the topic. Except for brief statements about the therapeutic indications of each drug, the book does not provide any therapeutic guidelines in different diseases, and was not intended to do so either. In such a case more information about drug effects and side-effects in infants and children would have been required.

Anders R[illegible]

Maureen Oswin. *Children living in long stay hospitals.* Spastics International Medical Publications (SIMP). William Heinemann Medical Books Ltd., London.

This book is not a clinical description of paediatric long stay patients, but deals with the situation of 223 seriously mentally retarded children from a psychological–social educational point of view. The children live in special care wards in eight English hospitals for retarded children. All the children have multiple handicaps, most of them have no spoken language or ability to move. You find a high percentage of extra handicaps as for example blindness and deafness. Judging from the descriptions the children must be seriously retarded.

According to the cover of the book the author is a teacher and has worked among retarded children si[nce] 1959. Already when seeing the title of the book you notice the interest and humane attitude of the author towards severely handicapped children. Children are children and have the same needs whether the diagnosis is a psychiatric or a physical one. Apart from special needs linked to the handicap, children in different handicap groups have common natural basic needs. Here the author is consistent and the children are simply called mentally handicapped.

The book has eight chapters which among other things describe the children, their families and special resources (physiotherapy, aids, speech therapy and some others). The need of medical consultants (paediatric and orthopaedic care) is also dealt with and the doctor is accused of not visiting the wards often enough and of not giving the child an adequate treatment. The book is full of illustrative examples.

The school situation of the children is dealt with in a special chapter. Compared to the situation in Sweden English special schools have less resources. In spite of

this they are well known for creativity and good results In one part of the book the situation of the nurses is described

There are two appendices and the first one Scenes in Ward 7 could very well be a first independent chapter Here Maureen Oswin describes everyday situations from the different wards in a dramatic and touching way Here you find things that the evening press could easily distort Maureen Oswin s description is objective and although the contents of the book as a whole is critical every chapter ends with constructive recommendations

There is a list of recommended further reading It is good but publications of James and Joyce Robertson are mi sing

The book is good reading for everybody who has to do with mentally handicapped children (medical consultants psychologists teachers nurses administrators etc) The book can be highly recommended and should be available in every hospital library

Per Vegfors

E L Kendig Jr & V Chern k (eds) *Disorders of the espi atory tract in child en* 3rd ed W B Saunders Company London 1115 pp illus £3 50 ISBN 0 7 16-5378

With Dr Victor Chernick as a new associate editor and twenty two new contributors the third edition of Edwin L Kendig s Disorders of the respiratory tract in children has become a still better textbook in paediatri pneumology than the former editions All chapters have been rewritten or been revised and brought up to date The seven new chapters dealing with important paediatric di orders have made this edition a very comprehensive book covering almost all paediatric di ea es of the lungs Most illustration including roentgenograms are excellent and all chapters are followed by a lot of up to date references Except some short chapters about disorders in adenoids and tonsils this edition is limited to disorders in the lower respiratory tract In spite of that it has become a large book containing sixty-eight chapters and almost 1100 pages It should be able to give answers to almost every clinical question about pulmonary diseases raised by physicians dealing with re piratory disorders in ch l dren

Hans Ahlst om

J V Simone (Guest editor) *Acute leukaemia In* Clinics in Haematology June 1978 00 pp illus tabl W B Saunders Company Ltd $16 50

This book deals in particular with the therapeutic aspects of acute leukaemia Clinical features cytology and infectious complications are not covered in this issue of Clinics in haematology

J V Simone has an excellent foreword with short comments on the topics new since the 197? edition on acute leukaemia It is also interesting to note his remarks on editorial selection bias

Five of the ten chapters are of particular interest to paediatricians Stephen L George points out the problems of design and evaluation of leukaemia trials A Mauer presents the principles of treatment of acute leukaemia in children and the current protocol studies from St Jude Children s Hospital The heterogeneity of the high risk group is stressed The important discovery of abnormal computerized tomography of the brains of children receiving CNS prophylaxis is mentioned and proposals for alternative CNS prophylaxis not including irradiation are briefly commented on All cases of AML off therapy at St Jude Children s Hospital have subsequently relapsed

Saunders and Donald Thomas from the Seattle group present an excellent survey and discussion of bone marrow transplantation a procedure characterized by Simone as an experimental treatment method bone marrow transplantation has so far been most disappointing in leukaemias in Europe

CNS and gonadal involvement are dealt with by Aur and Hustu in a chapter on extramedullary leukaemia (sanctuaries) The well known first 7 studies from St Jude Children s Hospital (196 –1971) are presented particularly with respect to their preventive CNS therapy The frequency of CNS relapse was 8/45 (17%) in cases receiving cranial irradiation+methotrexate i t in study VII and the median duration of haematological remission ?4 months (?3 months for craniospinal group)

The increasing incidence of testicular involvement is also mentioned (8 to 92% at autopsy) Prophylactic irradiation of the testes is not used at St Jude Children s Hospital and only one testis is irradiated in cases on unilateral leukaemic involvement

Peter Johan Moe

ANNOUNCEMENT

INTERNATIONAL WORKSHOP ON THE "AT RISK" INFANT

An international workshop on the "at risk" infant will be held in Tel Aviv, Israel, July 25–27, 30–31, 1979. The following topics will be discussed: Prospective parents, pregnancy and perinatal period, infancy and early childhood, standards for socio-ecological community health care facilities. For further information write to: The secretariat, P.O. Box 16271, Tel Aviv, Israel.

Acta Pædiatr Scand 68 469–473 1979

DEVELOPMENTAL PAEDIATRICS[1]

M MANCIAUX

In the sixties some authors in the States have described the syndrome of the dissatisfied paediatrician resulting from too much work without great intellectual and human interest

More recently the French paediatrician P Royer chairman of the International Paediatric Association elaborated on what he called the syndrom of the anxious paediatrician There are many reasons for this anxiety

- the zero growth of the population resulting in too few children
- the better health status of the children group as a whole
- the increase in number and improvement in quality of health structures at various levels
- the general practitioners wanting more and more to take care of children
- the multiplication of paediatric subspecialities and of the non medical professions dealing with children

What is left to paediatricians?

However in most developed countries

- perinatal care is still insufficient
- one does not know much on infant and child morbidity
- there is a gap of surveillance and care between one year of age and school entrance
- there are still shortcomings and delays in the diagnosis assessment and care of various handicapping conditions and chronic diseases
- attention and care for adolescents are badly lacking

These lacks and shortcomings plead for a more competent approach and interest by paediatricians

However most of these problems are psychosocial by nature Is not the key point that most paediatricians are poorly if at all prepared to this stimulating approach which is the privileged field of social or better psychosocial paediatrics?

Social paediatrics has undergone a tremendous development in many countries in the last 20 years but this does not reflect in the training of health and health related personnel Moreover in some countries it has become a kind of paediatric subspecialty which is a pity since all paediatrics should be preventive and social In other countries it has fragmented into many topics immunizations—public health related to childhood—child abuse and neglect—adolescent medicine—and so on losing the totality and the continuity of the children s development and welfare

That is the reason why I do prefer now tc use the concept and approach of our Britisl colleagues Developmental paediatrics enlarging it to encompass also the physica growth and the future of the child which is after all the main aim and outcome of growtl and development

In this concept developmental paediatric deals with

- physical growth and maturation
- psychosocial development
- screening for health and disease
- prevention in childhood of aging and bein later affected by degenerative diseases c old age

[1] Curt Gyllensward lecture given October 27 1978 , Stockholm

If this breakdown is useful for analysis it could be misleading as to the global view of paediatric problems We know how artificial it is to separate physical and psychosocial growth To speak only about nutrition which could be seen as a problem purely related to physical growth we are now aware of the ill effects on psychological development and school achievements, of too poor a diet during early life On the other hand we know the so called psychosocial dwarfism caused by the lack of stimulation of the child by his parents or caretakers So the reality of life is complex! However for making it easier we shall stick to our analytical approach starting with somatic development

In the field of *physical growth* developmental paediatrics should first include methods of collecting relevant and readily obtainable somatic growth data Among the problems to be clarified are

- the kinds of equipment to be used by the individual physician or paramedical workers
- which parameters are meaningful and usable
- the measuring techniques to be selected
- what kinds of graphs curves charts should serve as standards
- how often and at which ages should measurements be made (head chest arm)

Second more widespread and better use of these data is necessary for evaluation of the individual status of children e g the progress of a child born to parents who are either very tall or small can be interpreted correctly and differentiated from growth abnormalities due to inherited or acquired pathology e g a more suitable selection of sports physical exercises, may be provided for individual children after study of their biotype and their physical potentialities Moreover it could be of interest to correlate more systematically the problems of poor school achievements with biologic maturation status assessed for example by skeletal age measurement

Third applying this methodology to a large number of children using a well designed cross sectional protocol can result in the collection in a short period of time of valuable information on growth and development This information should be used on a public health basis for secular comparisons between generations and for contemporaneous comparisons of children in different socio economic groups and contexts

Furthermore growth data and charts derived from these data can be used for assessing the health of individuals and groups whether in private practice or in health centres and clinics The chart recently prepared by WHO for use in countries where no national references are available is a useful and appropriate tool Unfortunately developmental charts appear not to be used extensively by the paediatricians or public health workers either in the developing or developed world Developmental paediatrics should lead to a more accurate monitoring of children s growth and development both on an individual and a community basis It is especially important to control carefully the physical as well as the psychological growth and development of chronically ill children of children suffering from various kinds of handicaps etc in order to help them to achieve as far as possible their genetic potential of development that might have been affected by the disease and sometimes by the treatment

In the industrialized world *psychological development* is an area of child health which occupies or should occupy the attention of paediatricians to an increasing extent

Diagnosis and evaluation of psychosocial development is and probably will continue to be difficult and will require the close cooperation of medical and non medical disciplines These must include obstetrics paediatrics nutrition sciences psychology social work It is a real problem to fulfil the needs in this field when working with a large number of children in health centres In addition measuring mental and emotional maturation

levels is much more difficult than measuring physical growth The interpretation of psychosocial development may be hazardous and complicated

In passing one should recall the abuses to which the intellectual quotient has been subjected and the damage which has resulted for individuals groups and communities

Attempts to evaluate emotional and psychosocial development of children from the early age are the basis of developmental paediatrics Much effort has been made to set up standards for such an assessment and various scales have been prepared by specialists Using material from these workers for assessing at key periods the level of psychosocial development of children needs to be intensively taught and practiced The emphasis is now rightly put on the assessment and if necessary the reenforcement of the early mother/child relationship However other periods of life also deserve increasing attention

Throughout childhood and adolescence the main problems facing paediatricians in their daily practice pertain to the psychosocial field It is this area in which paediatric skills should be sharpened so that parents teachers psychologists can be guided when they have to deal with diagnosis and management of learning disabilities one of the commonest reasons for seeking paediatric help in the school age period

Later on during preadolescence parents are often at a complete loss as to the handling of many difficult or impossible children both at home and at school as indicated by the widespread but so far ineffective PINS programme in the States (Persons In Need of Supervision)

Because of inadequate or totally lacking solutions to these preadolescent problems there is a great risk that these children will become maladjusted adolescents In fact as clearly shown by data from all over the world delinquency is nowadays a problem of increasing concern and no solutions have been found so far which can be extensively applied

Drugs continue to spread among youth in most parts of the world In the States where the phenomenon appeared 20 years ago alcohol is the commonest teen age drug In a recent New York State schools survey between

60 and 90% of adolescents have been found using alcohol
40 and 60% of adolescents have been found using tobacco
6 and 40% of adolescents have been found using marijuana
5 and 15% of adolescents have been found using LSD
1 and 3% of adolescents have been found using heroin

The shifting from soft to hard drugs has been proved by several surveys For instance the Columbia University Research conducted in some schools of New York on a representative sample of adolescents from 14 to 18 years old has shown that

- 30% of youngsters who do not use any drug start alcohol and tobacco in the following 6 months
- 27% using alcohol and tobacco shift to marijuana
- 26% using marijuana try hard drugs after 6 months

Drug abuse in pregnancy has serious possible consequences for the passively exposed foetus since many of the preparations pass the placenta *readily Due* to the high rate of pregnancies in teen agers the effects of drug usage *by youth* on foetal health and growth and on labour have serious implications well studied in countries like Sweden It may be noted that already in 1971 more than 50% of deaths due to child abuse occurred in the States in families in which both parents were narcotic addicts

Screening is the third chapter of developmental paediatrics deriving quite naturally

If this breakdown is useful for analysis it could be misleading as to the global view of paediatric problems We know how artificial it is to separate physical and psychosocial growth To speak only about nutrition which could be seen as a problem purely related to physical growth we are now aware of the ill effects on psychological development and school achievements of too poor a diet during early life On the other hand we know the so called psychosocial dwarfism caused by the lack of stimulation of the child by his parents or caretakers So the reality of life is complex[1] However for making it easier we shall stick to our analytical approach starting with somatic development

In the field of *physical growth* developmental paediatrics should first include methods of collecting relevant and readily obtainable somatic growth data Among the problems to be clarified are

- the kinds of equipment to be used by the individual physician or paramedical workers
- which parameters are meaningful and usable
- the measuring techniques to be selected
- what kinds of graphs curves charts should serve as standards
- how often and at which ages should measurements be made (head chest arm)

Second more widespread and better use of these data is necessary for evaluation of the individual status of children e g the progress of a child born to parents who are either very tall or small can be interpreted correctly and differentiated from growth abnormalities due to inherited or acquired pathology e g a more suitable selection of sports physical exercises may be provided for individual children after study of their biotype and their physical potentialities Moreover it could be of interest to correlate more systematically the problems of poor school achievements with biologic maturation status assessed for example by skeletal age measurement

Third applying this methodology to a large number of children using a well designed cross sectional protocol can result in the collection in a short period of time of valuable information on growth and development This information should be used on a public health basis for secular comparisons between generations and for contemporaneous comparisons of children in different socio-economic groups and contexts

Furthermore growth data and charts derived from these data can be used for assessing the health of individuals and groups whether in private practice or in health centres and clinics The chart recently prepared by WHO for use in countries where no national references are available is a useful and appropriate tool Unfortunately developmental charts appear not to be used extensively by the paediatricians or public health workers either in the developing or developed world Developmental paediatrics should lead to a more accurate monitoring of children s growth and development both on an individual and a community basis It is especially important to control carefully the physical as well as the psychological growth and development of chronically ill children of children suffering from various kinds of handicaps etc in order to help them to achieve as far as possible their genetic potential of development that might have been affected by the disease and sometimes by the treatment

In the industrialized world *psychological development* is an area of child health which occupies or should occupy the attention of paediatricians to an increasing extent

Diagnosis and evaluation of psychosocial development is and probably will continue to be difficult and will require the close cooperation of medical and non medical disciplines These must include obstetrics paediatrics nutrition sciences psychology social work It is a real problem to fulfil the needs in this field when working with a large number of children in health centres In addition measuring mental and emotional maturation

levels is much more difficult than measuring physical growth The interpretation of psycho social development may be hazardous and complicated

In passing one should recall the abuses to which the intellectual quotient has been subjected and the damage which has re sulted for individuals groups and communi ties

Attempts to evaluate emotional and psycho social development of children from the early age are the basis of developmental paediatrics Much effort has been made to set up stand ards for such an assessment and various scales have been prepared by specialists Using material from these workers for assess ing at key periods the level of psychosocial development of children needs to be inten sively taught and practiced The emphasis is now rightly put on the assessment and if necessary the reenforcement of the early mother/child relationship However other periods of life also deserve increasing atten tion

Throughout childhood and adolescence the main problems facing paediatricians in their daily practice pertain to the psychosocial field It is this area in which paediatric skills should be sharpened so that parents teach ers psychologists can be guided when they have to deal with diagnosis and management of learning disabilities one of the commonest reasons for seeking paediatric help in the school age period

Later on during preadolescence parents are often at a complete loss as to the handling of many difficult or impossible children both at home and at school as indicated by the widespread but so far ineffective PINS programme in the States (Persons In Need of Supervision)

Because of inadequate or totally lacking solutions to these preadolescent problems there is a great risk that these children will become maladjusted adolescents In fact as clearly shown by data from all over the world delinquency is nowadays a problem of increasing concern and no solutions have been found so far which can be extensively applied

Drugs continue to spread among youth in most parts of the world In the States where the phenomenon appeared 20 years ago alcohol is the commonest teen age drug In a recent New York State schools survey between

60 and 90% of adolescents have been found using alcohol
40 and 60% of adolescents have been found using tobacco
6 and 40% of adolescents have been found using marijuana
5 and 15% of adolescents have been found using LSD
1 and 3% of adolescents have been found using heroin

The shifting from soft to hard drugs has been proved by several surveys For instance the Columbia University Research conducted in some schools of New York on a repre sentative sample of adolescents from 14 to 18 years old has shown that

- 30% of youngsters who do not use any drug start alcohol and tobacco in the following 6 months
- 27% using alcohol and tobacco shift to marijuana
- 26% using marijuana try hard drugs after 6 months

Drug abuse in pregnancy has serious pos sible consequences for the passively exposed foetus since many of the preparations pass the placenta readily Due to the high rate of pregnancies in teen agers the effects of drug usage by youth on foetal health and growth and on labour have serious implications well studied in countries like Sweden It may be noted that already in 1971 more than 50% of deaths due to child abuse occurred in the States in families in which both parents were narcotic addicts

Screening is the third chapter of develop- mental paediatrics deriving quite naturally

from the observation of physical and psychosocial growth and development

To elaborate on screening would take too long! Let us simply consider some theoretical points related to screening in its principle and practice

According to WHO screening is the presumptive identification of unrecognized disease or defects by the application of tests examinations or procedures which can be applied rapidly One could also add safely and at low cost at least for mass screening In many cases selective screening is more valuable and cost/benefit acceptable Based on epidemiological studies it aims at identifying causative factors and determining at risk/at high risk groups through sound and feasible indicators factors and indicators on which a screening procedure can be built

Screening was first developed for disease *The criteria to be fulfilled are as follows* The disease is

- serious
- possible to diagnose
- treatable either medically or by rehabilitation *and the prognosis is improved through* early treatment
- there is an adequate screening period
- the disease is relatively prevalent
- the screening procedure is not harmful
- *facilities are available for screening evaluation* and treatment
- the procedure is of reasonable cost and
- acceptable to the population

(Frankenburg 1976)

Selection and use of *screening* tests and procedure must consider

- prevalence and natural history of the disease
- target population
- availability and cost of screening
- definitive evaluation of positive patients

(M Gutgesell 1978)

The future of screening lies in a better fulfilment of criteria (in terms of cost/benefit cost/efficiency) in screening for handicapping conditions The full process of screening assessment and rehabilitation should be established Finally the screening procedure must be integrated into the full range of health services

However the foreseeable future of screening will be to screen not only for disease but also for health and we should encourage the use of development screening tests by primary care paediatricians

There again the practice is far from being satisfactory For example a survey conducted in the USA has shown that paediatricians use development screening tests infrequently and usually only after evidence of development delay has been established by *other criteria* We are far from the concept of screening for development and health as elaborated by Mary Sheridan and Ronnie McKeith in the early sixties!

Paediatrics *is by nature preventive* since each single care or cure intervention in childhood is aimed at safeguarding or restoring the health of the child not only for the present but also for a better future

Moreover with the new knowledge concerning the possibilities of prevention of certain adult diseases based on biochemical and physiologic data derived from cross sectional studies paediatrics becomes not only preventive but really *prospective* This is the most fascinating aspect of developmental paediatrics based on scientific research as well as on carefully planned epidemiological surveys

In 1974 a WHO consultative group reviewed the then current research related to the early possible childhood antecedents of atherosclerosis *It stressed the need for* continued studies in this field and listed a number of problems which require elucidation

Some risk factors were outlined which might suggest a risk pattern Screening for these should be done in early childhood and should consist of data on family history biochemical findings blood pressure weight physical activity *cigarette smoking etc*

Half of the 8 or 10 risk factors related to coronary heart disease may be induced in childhood most of them are closely allied with the way of living especially with nutritional habits However their degree of significance their persistence through adolescence and adulthood their combination in a risk pattern their potential dangers have not as yet been fully documented This means that it is probably too soon to develop a body of preventive guidelines

Many studies are under way some of them under WHO auspices which may help clarify this important public health problem Meanwhile one can only attempt to prevent or treat especially in families at risk such harmful factors as overnutrition obesity hypertension cigarette smoking etc

Closing a British symposium entitled Prevention of coronary heart disease starts in childhood O Wolff turned to the educative role of the paediatrician towards colleagues in the health and teaching fields who are in the best position to influence children and their families in making changes in eating and other habits Paediatricians should seek to show these groups the importance of starting good habits in childhood This is also developmental paediatrics[1]

ACKNOWLEDGEMENT

I wish to thank Dr Samuel Berenberg of the I C C for having read the manuscript during its preparation and for some of the suggestions he made as to language and content

International Children s Centre
Chateau de Longchamp
Carrefour de Longchamp-Bois de Boulogne
Paris XVI
France

Acta Pædiatr Scand 68 475–479 1979

ATTEMPT AT ENZYME REPLACEMENT IN GAUCHER DISEASE BY RENAL TRANSPLANTATION

C G GROTH H COLLSTE S DREBORG G HÅKANSSON G LUNDGREN and L SVENNERHOLM

From the Division of Transplantation Surgery Department of Surgery Karolinska Institutet Stockholm Huddinge Hospital Huddinge the Department of Neurochemistry University of Gothenburg and the Department of Paediatrics County Hospital Boden Sweden

ABSTRACT Groth C G Collste H Dreborg S Håkansson G Lundgren G and Svennerholm L (Division of Transplantation Surgery Department of Surgery Karolinska Institute Stockholm Huddinge Hospital Huddinge the Department of Neurochemistry University of Gothenburg Gothenburg and the Department of Paediatrics County Hospital Boden Sweden) Attempt at enzyme replacement in Gaucher disease by renal transplantation Acta Paediatr Scand 68 475 1979 —In Gaucher disease there is a deficiency of the lysosomal enzyme cerebroside-β-glucosidase as a result of which cerebroside (glucosyl ceramide) accumulates in various organs In northern Sweden 22 patients with a juvenile form of this disease have been identified In one such patient a girl of 10 years we have attempted enzyme replacement by renal transplantation After this operation the hepatic glucocerebroside content fell significantly In another child afflicted with Gaucher disease in whom splenectomy was performed for severe splenomegaly and hypersplenism there was a progressive increase in the level of this lipid These findings suggest that enzyme replacement was achieved by transplantation of a normal organ

KEY WORDS Gaucher disease enzyme replacement renal transplantation

In a patient suffering from an enzyme deficiency disease enzyme replacement could conceivably be achieved by transplantation of a normal organ In Gaucher disease there is a deficiency of the lysosomal enzyme cerebroside β glucosidase as a result of which cerebroside (glucosylceramide) accumulates in various tissues and organs especially the spleen liver and skeleton In northern Sweden 22 patients with a distinct genetic form of the disease have been identified (5 6) Although the time of onset and the course of the disease show considerable variations the majority of the patients suffer from a juvenile form of the disease Early on massive deposits of cerebroside appear in the spleen splenectomy leads to accelerated cerebroside storage in the liver and skeleton and symptoms from the central nervous system (5 6)

In one splenectomized child we have attempted enzyme replacement by renal transplantation having first ascertained that renal tissue contained a high level of cerebroside β glucosidase After the operation the hepatic cerebroside concentration was determined at intervals The same studies were carried out in another Gaucher patient of the same age who underwent splenectomy for severe splenomegaly and hypersplenism but received no transplant

MATERIAL AND METHODS

Enzyme and lipid assays

The cerebroside β-glucosidase activity in leukocytes and parenchymatous organs was measured with glucose [C]-cerebroside as a substrate (1) With this method the enzyme activity in leukocytes from normal subjects is 7 69±0 64 μkat/kg of protein in our laboratory

The splenic and hepatic cerebroside concentrations were examined by thin layer chromatography and densitometric scanning of the charred cerebroside spot using cerebroside isolated from a Gaucher patient as a standard (?) In the liver and spleen from normal individuals the cerebroside content is less than 0 03 μmol/g of fresh

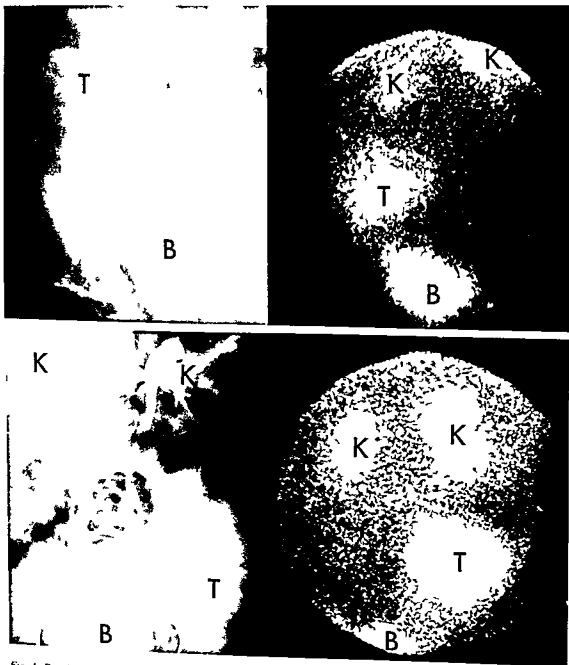


Fig 1 Renal transplants in a patient with Gaucher disease Second (*upper*) and third (*lower*) renal grafts visualized with intravenous pyelography and radio isotope scintigraphy Note the normal function of the transplants (*T*) and of the patient s own kidneys (*K*) The bladder (*B*) is also visualized

and scintigraphy showed excellent graft function (Fig 1) Three weeks later there was a deterioration of function and the prednisone dose was increased Five weeks after the operation high fever and sekeletal pains developed followed by dyspnoea and hypoxia Antibiotics were given and the doses of the immunosuppressive drugs were reduced Despite these measures diffuse pulmonary infiltration developed and death from hypoxia

tissue The phospholipid content which was used as an indicator for the tissue s state of hydration was estimated as inorganic phosphate after wet ashing (9)

In the 2 Gaucher patients liver specimens were obtained at intervals by open biopsy The tissue was frozen immediately in liquid nitrogen and kept frozen until analysis The frozen specimen was cut into thin sections weighing 10–20 mg each of which was submitted to analysis Assays were performed on 5–20 pieces from each specimen The assays on the specimens from the transplant recipient were performed on a single occasion after storage for up to 1 year Although the specimens were wrapped in polyethylene film there had been a significant loss of water as evidenced by elevated and variable levels of liver phospholipid In these specimens the best measure of changes in cerebroside content is provided by the cerebroside/phospholipid molar ratio The assays on the biopsy specimens from the splenectomized patient were all performed without delay

Normal tissue for measurement of enzyme activity

Cadaveric renal hepatic and splenic tissue was harvested from 12 non-Gaucher subjects (aged 40–80 years) The specimens were obtained between 5 minutes and 12 hours after death and kept frozen until examined for cerebroside β glucosidase activity

Patient undergoing renal transplantation

This was a 10 year-old girl with a known familial history of Gaucher disease In infancy hepatomegaly splenomegaly and pancytopenia had been diagnosed At 6 years the large spleen (1 0 kg) had been removed The cerebroside content was 44 9 μmol/g of fresh tissue (cerebroside/phospholipid ratio 2 96 1)

At 8–10 years skeletal destruction had developed which resulted in multiple spontaneous fractures and severe skeletal pain There was moderate mental retardation On admission to this Hospital the hepatomegaly was at an advanced stage and biopsy showed the presence of numerous Gaucher cells The cerebroside β glucosidase activity in the leukocytes was 0 17–0 32 μkat/kg of protein (6–12% of the normal activity)

Patient undergoing splenectomy

This was a 10 year-old boy with a known familial history of Gaucher disease One of 4 sibs has died from the disease the other 2 were not afflicted When the boy was 2 years of age Gaucher cells were identified in a splenic biopsy specimen There was both hepato- and splenomegaly At 9 years skeletal destruction typical of the disease developed The cerebroside β glucosidase activity in the leukocytes was 0 48 μkat/kg (18% of the normal activity) At 10 years the spleen was so large as to greatly interfere with the child s mobility and there was thrombocytopenia with bleeding

RESULTS

Enzyme activity in normal tissues

The mean cerebroside β glucosidase activities in the non Gaucher renal hepatic and splenic tissue were found to be 9 9±2 1 (S D) 4 1±2 1 and 4 7±2 1 μkat/kg of protein respectively

Patient undergoing renal transplantation

In August 1975 a cadaveric kidney was transplanted from a child of the same age as the recipient There were 2 HLA A B incompatibilities The vascular anastomoses were to the right iliac vessels and the ureter was implanted into the bladder The immunosuppressive agents were azathioprine and prednisone As the patient s own kidneys functioned normally the condition of the graft could be appraised only by radiological examination and urine analysis

Eight days after the operation there was evidence of acute rejection with fever proteinuria and an elevated amount of fibrinogen degradation products in the urine The transplant was not visualized on scintigraphy or intravenous pyelography angiography revealed vascular abnormalities consistent with rejection

On day 11 the transplant was removed and replaced by a cadaveric kidney from another child of the same age (3 HLA A B incompatibilities) Microscopic examination of the first graft confirmed acute rejection

The second transplant produced large volumes of urine immediately after revascularization There was again an early acute rejection episode but this was reversed with prednisone One month after transplantation the graft was excellently visualized by pyelography and scintigraphy (Fig 1) During the next 7 months the clinical condition improved the skeletal pains were less severe and mobility was increased

Seven months after the operation fever developed The graft was not visualized radiographically On exploration it was found to be rejected and thrombosed and it was removed

At the same operation a cadaveric kidney from an adult (2 HLA A B incompatibilities) was anastomosed to the iliac vessels on the left side Diuresis was immediate and pyelography

ticularly by the liver cells where they would then degrade the stored cerebroside

In the child undergoing splenectomy there was a progressive increase in the hepatic glucocerebroside content after the operation This finding indicates that the spleen is a major reservoir for the cerebroside that the patient is unable to degrade when the spleen is removed there will be a rapid increase in the liver s cerebroside content Increasing deposits in other organs will probably also occur previously studied patients have displayed increasing skeletal involvement and mental retardation in the years after splenectomy (6) This suggests that any attempt at enzyme replacement therapy in Gaucher disease should be made at the time of splenectomy or soon afterwards

Organ transplantation clearly holds promise as a means of enzyme replacement The greatest impediment to lasting success is the destruction of the transplant by rejection

REFERENCES

1 Brady R O Kanfer J N & Shapiro D The metabolism of glucocerebrosides I Purification and properties of a glucocerebroside-cleaving enzyme from spleen tissue *J B ol Chem* ?40 39 1965

Brady R O Pentchew P G Gal A E Hibbert S R & Dekaban A S Replacement therapy for inherited enzyme deficiency Use of purified glucocerebrosidase in Gaucher s disease *N Engl J Med 291* 889 1974

3 Desnick S J Desnick R J Brady R O Pentchew P G Simmons R L Najarian J S Swaiman K Sharp H L & Krivit W Renal transplantation in type II Gaucher s disease *In* D Bergsma (ed) Enzyme therapy in genetic disease *Birth Defects Orig Art Series* 9 10? 1973

4 Groth C G Hagenfeldt L Dreborg S Lofstrom B Öckerman P A Samuelsson K Svennerholm L Werner B & Westberg G Splenic transplantation in a case of Gaucher s disease *Lancet i* 1760 1971

5 Herrlin K M & Hillborg P O Neurological signs of a juvenile form of Gaucher s disease *Acta Pediatr Scand 51* 137 196?

6 Hillborg P O & Svennerholm L Blood level of cerebrosides in Gaucher s disease *Acta Pediatr Scand 49* 707 1960

7 Neufeldt E F Sando G N Garvin A J & Rome L H The transport of lysosomal enzymes *J Supramol Struct 6* 95 1977

8 Schlesinger P Rodman J S Frey M Lang S & Stahl P Clearance of lysosomal hydrolases following intravenous infusion The role of liver in the clearance of β glucuronidase and N acetyl β D-glucosaminidase *Arch Biochem Biophys 177* 606 1976

9 Svennerholm L & Vanier M Th The distribution of lipids in the human nervous system II Lipid composition of human fetal and infant brain *Brain Res 47* 457 197?

Submitted Nov 2? 1978
Accepted Jan 19 1979

(C G G) Division of Transplantation Surgery
Huddinge Hospital
S 141 86 Huddinge
Sweden

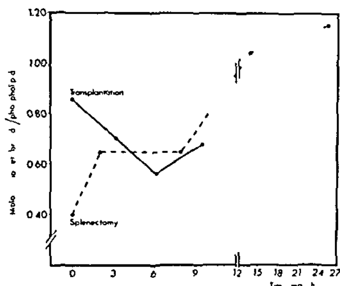


Fig 2 The hepatic cerebroside content in 2 patients with Gaucher disease one undergoing renal transplantation for enzyme replacement the other splenectomy because of severe splenomegaly * denotes a significant change since the previous reading

ensued 2 months after the third transplantation At autopsy cytomegalovirus was isolated from the lung tissue The renal transplant showed histologic changes indicative of mild chronic rejection

Over the 7 months during which the second renal graft was functioning the glucocerebroside level in the recipient s liver fell from 44 0 to 24 3 μmol/g of tissue and the cerebroside/phospholipid molar ratio fell from 0 86 to 0 57 In the post mortem hepatic tissue the lipid had increased to 30 3 and the ratio to 0 73 (Fig 2) The cerebroside β glucosidase activity in the removed first graft was within the normal range for renal tissue at 9 1 μkat/kg

Patient undergoing splenectomy

Splenectomy was performed in April 1976 The enormous spleen weighed 2 4 kg The cerebroside content was 33 2 μmol/g net weight of tissue (cerebroside/phospholipid ratio 1 85) After the operation there was an improvement in the patient s mobility and the platelet count was normalized Up to the time of this report 2 years after splenectomy the child s general condition has remained satisfactory but he has had at least one episode of bone necrosis with infarction of the femoral neck

Since splenectomy the hepatic glucocerebroside level has risen from 12 9 μmol to 34 8 and the cerebroside/phospholipid ratio from 0 40 to 1 15 (Fig 2)

DISCUSSION

We have previously attempted a splenic transplantation in a patient with juvenile Gaucher disease (4) During the 7 weeks that the graft was functioning there was indirect evidence of a beneficial metabolic effect with a reduction in the serum level of acid phosphatases An attempt at renal transplantation to a child with infantile Gaucher disease by Desnick et al was followed by a temporary clinical improvement (3)

It was formerly supposed that the cerebroside β glucosidase activity is particularly high in splenic tissue Our knowledge of the activity of the enzyme in various organs has however been incomplete Our own data led us to attempt to supplement the enzyme by renal transplantation

The reduction in the cerebroside content of the liver in the patient carrying a renal transplant suggests that enzyme replacement was accomplished The reason for the metabolic and clinical deterioration observed during the last few months is unclear One possibility is that the infection from which the patient was suffering provoked an exacerbation of the metabolic disease

Neufeld and co workers have recently proposed that hydrolytic enzymes are introduced into lysosomes only after scretion and receptor mediated capture these mechanisms apparently operate in cultured fibroblasts (7) Lysosomal enzymes injected intravenously into rats are rapidly cleared from the circulation—mainly into the liver and spleen (8) It is tempting to speculate that the transplant will secrete lysosomal enzymes and that these would be taken up by various cells and par

Acta Pædiatr Scand 68 481–483 1979

THE PROTECTIVE EFFECT OF ACUTE PHASE REACTANTS IN NEONATAL SEPSIS

ALISTAIR G S PHILIP

From the Department of Pediatrics University of Vermont College of Medicine Burlington Vermont USA

ABSTRACT Philip A G S (Department of Pediatrics University of Vermont USA) The protective effect of acute phase reactants in neonatal sepsis Acta Paediatr Scand 68 481 1979.—Phase reactants were evaluated prospectively in babies suspected of having sepsis Among 318 babies there were 2? proven and 10 very probable cases of neonatal sepsis Of the proven cases 14 survived and 8 died The survivors had a positive latex C reactive protein (CRP) in 11 cases and an alpha$_1$-acid glycoprotein (AGP) level greater than 0 5 g/l in 1 cases Among those who died one had a positive latex CRP and none had AGP >0 5 g/l These findings were supported by positive CRP and elevated AGP in almost all very probable cases all of whom survived These data in newborn infants support the hypothesis that acute phase reactants have a functional role in combating infection

KEY WORDS Newborn sepsis acute phase reactants

In the presence of inflammation or infection the concentration of a number of serum proteins increases as the acute phase response The evaluation of these acute phase reactants in the newborn period has been primarily directed at the diagnosis of infection Acute phase reactants which have been evaluated in neonatal sepsis include C reactive protein (8) haptoglobin (9) α_1 acid glycoprotein (orosomucoid) (3 10) fibrinogen and other proteins which influence the erythrocyte sedimentation rate (1 7)

Little attention has been paid to the *function* of these acute phase proteins C reactive protein has been described as a phagocytosis promoting factor (4) Recent experimental evidence indicates that in adults both C reactive protein (5) and α_1 acid glycoprotein (2) may play an important role in modifying lymphocyte responsiveness

This report provides evidence in newborn infants which supports the hypothesis that acute phase reactants may be important in helping to combat infection

MATERIALS AND METHODS

Acute phase reactants were evaluated in a prospective study of neonatal sepsis during the first week of life (6) Serum was obtained from 318 babies who were being investigated with a "sepsis work up for either risk factors (e g prolonged rupture of membranes smelly amniotic fluid maternal fever etc) or clinical manifestations of neonatal sepsis (e g lethargy abdominal distension temperature instability etc)

There were 22 babies with proven bacterial sepsis who provide the main focus of this report Several diagnostic tests for sepsis were used including C reactive protein (CRP) and α_1 acid glycoprotein (AGP)

C reactive protein was measured with a latex reagent method which provides a positive reaction with levels equal to or greater than 8 mg/l

Alpha acid glycoprotein (orosomucoid) was measured with an immunodiffusion method [1]

Low birth weight (LBW) is defined as less than 2500 g

RESULTS

Of the 22 babies with proven bacterial sepsis 14 babies survived (7 LBW) and eight babies

---

[1] Reagents kindly supplied by Behring Diagnostics Somerville New Jersey

DISCUSSION

Evaluation of babies with proven bacterial sepsis showed that elevated levels of two proteins considered to be acute phase reactants were strongly correlated with mortality Those newborn infants who had elevated levels of C reactive protein and α_1 acid glycoprotein when sepsis was first suspected survived Those with low levels of CRP and AGP usually died These findings strongly support the hypothesis that both CRP and AGP play an important role in the ability to combat infection

Although there appears to be a strong influence of birth weight upon survival or death from neonatal sepsis there were several notable exceptions It seems more likely that the very premature (preterm) newborn infant has difficulty with or is sometimes incapable of mounting an appropriate response to bacterial infection When a baby is able to respond appropriately by producing (increasing) acute phase reactants there appears to be a protective effect

The findings in the cases of proven bacterial sepsis support the hypothesis of a protective effect of acute phase reactants Further support is provided by the elevated levels of CRP and AGP found in the cases of very probable infection all of whom survived Elevated levels are infrequently seen when there is no evidence of infection The values for CRP indicate that a positive response is not determined entirely by birth weight Less than 10% of babies weighing more than 2 500 g had a positive test and several very LBW babies were able to produce an increase in CRP The mean level for AGP in heavier babies was not dissimilar to the total group

The function of acute phase reactants has been speculative until recently Evidence in vitro has indicated the importance of CRP and AGP in modulating lymphocytes (2 5) CRP increased in vitro phagocytosis of *D pneumoniae Staph aureus E coli* and *Klebsiella aerogenes* (4) The evidence presented here strongly suggests that in newborn infants acute phase reactants also have a protective effect against neonatal sepsis

REFERENCES

1 Adler S M & Denton R L The erythrocyte sedimentation rate in the newborn period *J Pediatr* 86 94? 1975

2 Chiu K M Mortensen R F Osmand A P & Gewurz H Interactions of alpha acid glycoprotein with the immune system I Purification and effects upon lymphocyte responsiveness *Immunology 32* 997 1977

3 Gotoh H Ishikawa N Shion T Hattori Y Nomura H & Ogawa J Diagnostic significance of serum orosomucoid level in bacterial infections during neonatal period *Acta Paediatr Scand 62* 6 9 1973

4 Kindmark C-O Stimulating effect of C reactive protein on phagocytosis of various species of pathogenic bacteria *Clin Exp Immunol 8* 941 1971

5 Mortensen R F & Gewurz H Effects of C reactive protein on the lymphoid system II Inhibition of mixed lymphocyte reactivity and generation of cytotoxic lymphocytes *J Immunol 116* 1?44 1976

6 Philip A G S & Hewitt J R Early diagnosis of neonatal sepsis (Submitted for publication)

7 Reher J P Toubas P L & Minkowski A Elevated fibrinogen in the diagnosis of bacterial materno-fetal infection *In* L Stern B Friis-Hansen and P Kildeberg (eds) *Intensive care in the newborn* Masson Publishing Co New York 1976 p 51

8 Sabel K G & Hanson L Å The clinical usefulness of C reactive protein (CRP) determinations in bacterial meningitis and septicemia in infancy *Acta Paediatr Scand 63* 381 1974

9 Salmi T T Haptoglobin levels in the plasma of newborn infants with special reference to infections *Acta Paed at Scand* Suppl ?41 1973

10 Sann L Krawiecki N Bourgeois J & Hermier M Etude de l'orosomucoide chez le nouveau ne interet dans le diagnosti des infections bacteriennes *A ch Fr Pediatr 33* 961 1976

Submitted Oct 16 1978
Accepted Dec 13 1978

Department of Pediatrics
Given Medical Building
University of Vermont College of Medi ine
Burlington Vermont 05401
USA

Table 1 *Outcome in babies with neonatal sepsis related to the presence or absence of a positive latex C reactive protein test*

Latex CRP	Survived	Died	Mean birth weight (g)
−ve	3	7	1 789
+ve	11	1	2 423

Table 2 *Outcome in babies with neonatal sepsis related to the level of α_1 acid glycoprotein (orosomucoid)*

α_1 AGP (g/l)	Survived	Died	Mean birth weight (g)
<0 5	2	8	1 780
>0 5	12	0	2 431

died (7 LBW) The babies were classified according to whether they lived or died and with respect to C reactive protein (Table 1) and α_1 acid glycoprotein (Table 2) It is clear that when either of these two phase reactants was present in significant amount the likelihood of survival was very good When low levels of phase reactant were present death from infection was likely

The mean birth weight of the survivors was 2 517 g versus 1 466 g for those who died However it is pertinent to point out that one infant with birth weight 1 180 g had a positive CRP and AGP of 1 44 g/l and survived and another infant with birth weight 2 665 g had a negative CRP and AGP of 0 15 g/l and died of infection It is also noteworthy that all of 10 infants with clinical features strongly suggestive of sepsis but without a positive blood culture survived Nine had AGP greater than 0 5 g/l and eight had a positive CRP (Table 3)

Evidence that birth weight may not be the major determinant is provided by the following facts Babies with weights over 2 500 g without evidence of infection (n=89) had a mean level of AGP of 0 324 g/l compared to a mean level of 0 265 g/l for the total group without evidence of infection (n=281) There were 33 babies in this latter group who had AGP levels greater than 0 5 g/l Of these 12 babies were LBW CRP was positive in 19 babies without evidence of infection Eleven of the 19 babies were low birth weight The smallest baby with a positive test weighed 1 020 g

Table 3 *Findings in 10 babies who had strong presumptive evidence of infection but lacked a positive blood culture*

S W U = sepsis work up G B S = Group B Beta hemolytic streptococcus

Identity no	Age at S W U	Birth weight (g)	Sex	CRP	α_1AGP (g/l)	Factors suggesting infection
1/16	2 d	3 544	M	+ve	0 64	Pneumonia
1/41	8 h	2 041	M	−ve	0 84	W B C = 2 300/mm³ pneumonia maternal G B S
1/104	12 h	2 840	M	+ve	0 39	Lethargy tracheal aspirate—G B S
1/112	? d	4 111	M	+ve	0 66	Fever pustule grew staph aureus coag positive
1/166	4 d	2 280	M	−ve	0 67	Poor feeding shock W B C = 3 200/mm³
2/40	2 d	2 060	F	+ve	0 58	Pneumonia tracheal aspirate—G B S
2/46	4 h	2 980	F	+ve	0 93	Apnea shock double dose antibiotics I V before transfer and before blood culture
2/99	6 h	3 232	F	+ve	0 91	Fever maternal G B S
?/114	4 h	4 000	M	+ve	0 96	Maternal fever and antibiotics tracheal aspirate—E coli antibiotics I V before transfer and before blood culture
2/120	3 d	2 395	F	+ve	>2 0	Pneumonia fever

Acta Pædiatr Scand 68 485–493 1979

LISTERIOSIS DURING PREGNANCY AND NEONATAL PERIOD IN SWEDEN 1958–1974

S LARSSON S CRONBERG and S WINBLAD

From the Departments of Infectious Diseases and Bacteriology University of Lund General Hospital Malmo Sweden

ABSTRACT Larsson S Cronberg S and Winblad S (Departments of Infectious Diseases and Bacteriology University of Lund General Hospital Malmo Sweden) Listeriosis during pregnancy and neonatal period in Sweden 1958–1974 Acta Paediatr Scand 68 485 1979 — In 1958–1974 altogether 46 cases of bacteriologically verified infection of Listeria monocytogenes were diagnosed during pregnancy and the neonatal period Eight pregnancies resulted in abortion and three in stillbirth Thirty seven children were born alive 17 of whom died all but one within a few days These children were divided into three groups according to their age at the onset of illness 22 cases with Early disease (≤2 days) four cases with Intermediate disease (3–5 days) and eight cases with Late disease (≥6 days) Three children were apparently healthy Septicemia or Granulomatosis infantiseptica dominated in Early disease and claimed as many as 13 deaths In Late disease all the children had meningo-encephalitis and only one of them died The symptoms were typical of purulent meningitis The group of Intermediate disease consisted of overlapping cases of the other two groups Ampicillin alone or combined with gentamicin seemed to be the drug of choice in the therapy of neonatal listeriosis Of the surviving children two were seriously damaged and two had moderate injuries Fifteen children are apparently healthy In cases where pregnancy terminated in abortions stillborns or children with Early disease the mothers often showed signs of infection The mothers of the children with Late disease were apparently healthy These children were infected from other sources some of them nosocomially If listeriosis is diagnosed during pregnancy the women should be treated with ampicillin

KEY WORDS Listeria monocytogenes pregnancy neonatal period abortion stillborn septicemia meningo-encephalitis infection in utero nosocomial

Pregnant women and newborns are predisposed to infection with Listeria monocytogenes (L m) and listeriosis during pregnancy and the neonatal period is a common manifestation of the disease (3 5 24) The reason why the pregnant woman is susceptible is obscure but as a rule she is not seriously affected When the foetus or the newborn is infected listeriosis often causes a serious infection with a high mortality The first case of human infection diagnosed in Sweden was a boy with Granulomatosis infantiseptica reported in 1958 (19) This prompted Winblad (19) to register all bacteriologically verified cases of listeriosis in Sweden The National Health Service made listeriosis notifiable as from 1960 Part of this material—64 cases of listeriosis in children more than 27 days old and adults—has been published separately (17) The observations made in 46 pregnant women and their neonates are reported below

MATERIAL AND METHODS

Patients All cases of listeriosis diagnosed in pregnant women and newborns up to 27 days of age registered in 1958–1974 were included The diagnosis had been verified by isolation of the microorganism in 20 cases from the women in 4? cases from the foetuses/neonates and in 15 cases from both of them L m was isolated from the urethra cervical and vaginal secretion placenta amniotic fluid membranes blood or faeces from the women and from cerebrospinal fluid (CSF) blood throat nose eye pustules and from various organs obtained at necropsy of the foetuses/neonates

Table 3 *Neonatal listeriosis in Sweden 1958–1974 34 cases*

	Early disease (≤[illegible] days)	Intermediate disease (3–5 days)	Late disease (≥6 days)
Septicemia	19/[illegible]	[illegible]/4	0/8
Meningitis	3/[illegible]	[illegible]/4	8/8
Mortality	13/[illegible]	[illegible]/4	1/8
Maternal fever	9/[illegible]	1/4	1/8
Discoloration of amniotic fluid	16/[illegible]1	1/3	1/5
L m pos culture in mother	10/14	1/[illegible]	0/4
L m pos culture in child	22/[illegible]	4/4	8/8
Birth weight g (median)	[illegible] 440		3 460
Gestational age weeks (median)	37		38
Pos serology for listeria	15/16	1/3	0/3
Complicated parturition	8/[illegible]	0/4	1/8

O-agglutiter >1/40 and/or CF titer >1/10

(Table 3) The median birth weight was significantly higher in this group than in the group of Early disease (Rank sum test $p<0.05$) The mortality was low

Most of the children with septicemia were in a bad condition already at birth Nineteen had signs of respiratory distress and circulatory failure Seventeen children had neurological symptoms mostly flaccidity or increased tone with twitching Seizures occurred in eight of these children seven of whom died All four children with petechiae died Vomiting and/or diarrhoea occurred in four children and enlargement of the liver in six Nine out of 17 children studied got fever and eight children including five with a birth weight of less than 2 500 g had a low or normal temperature

In the cases of meningo-encephalitis the symptoms were typical of purulent meningitis with fever irritability whimpering and sometimes loss of appetite A tense fontanelle was noted in six cases Six children had seizures which was fatal in one Four children had respiratory symptoms five had vomiting and/or diarrhoea and five children had enlargement of the liver All children except one weighing less than 2 500 g had fever

In the primary CSF the number of polymorphonuclear as well as of mononuclear cells was increased (Table 4) Polymorphonuclear cells dominated in eight children and mononuclear cells in five Protein in the CSF was substantially increased (Table 4) and the glucose concentration was decreased in only three out of five cases studied

Antimicrobial treatment Since these patients had been treated in different hospitals there was no uniformity of the chemotherapeutic treatment for which reason caution should be exercised in the evaluation of the

Table 4 *Findings in primary CSF in meningo encephalitis in neonatal listeriosis in Sweden 1958–1974 13 cases*

	No of cases	Median value	Range
Total number of cells per l	13	3 630×10[illegible]	198–6 600×10
Polymorphonuclear per l	11	1 963×10[illegible]	23–3 400×10[illegible]
Mononuclear per l	11	1 000×10[illegible]	[illegible]63–3 76[illegible]×10[illegible]
Protein/g per l	10	[illegible] 0	1 9–10 [illegible]4
Polymorphonuclears in relation to mononuclears	13	Poly > Mono 8	Mono > Poly 5



Fig 1 Annual incidence and outcome of neonatal listeriosis in Sweden 1958–1974 46 women (2 pairs of twins) ■ = abortions □ = livebirths (survivors) ▨ = stillborns ▥ = livebirths (succumbed later)

Table 2 *Abortions and stillborns in listeriosis in Sweden 1958–1974 11 cases*

Abortion	8/11
Stillborn	3/11
Maternal fever	5/11
Discoloration of amniotic fluid	2/3
L m pos culture in mother	8/9
L m pos culture in foetus	8/8
Serology positive	6/8

O aggl titer >1/40 and/or CF titer >1/10

Clinical information was obtained from a study of all patients records and was sometimes supplemented by direct information from the patients and their relatives or from members of the hospital staff

Part of this material has been published earlier as case reports (2 6 8 9 10 12 14 19 21 22 26 27) Seasonal variation geographic distribution animal contacts serotypes and other epidemiological aspects are reported elsewhere (15) The pathological changes found at necropsy are published separately (18)

Bacteriology The technique used for bacteriological culture and diagnosis of Listeria monocytogenes has been described elsewhere (15)

Serology Antibodies against L m were assayed according to Winblad (15 28)

RESULTS

After the discovery of the first case in 1958 there were rather many cases in 1959 and 1960 Since then the frequency has been rather even and low (Fig 1)

Clinical manifestation

Eight pregnancies ended in abortion and three in stillborn foetuses (Table 1) Thirty seven children were born alive and 21 of these had septicemia a name we prefer to the earlier designation Granulomatosis infantiseptica

Table 1 *Listeriosis during pregnancy in Sweden 1958–1974*

Clinical diagnosis	46 women (two pairs of twins)
Abortion	8
Stillborn	3
Children born alive	
Granulomatosis infantiseptica	21 (15 fatal)
Meningo-encephalitis	13 (1 fatal)
No listeria infection	3 (1 fatal)

which proved fatal in 15 Thirteen children had meningo-encephalitis which was fatal in one Three of the infected women delivered children with apparently no clinical or bacteriological signs of listeriosis One of these children was born in the 24th week of pregnancy and soon died because of its immaturity

Clinical course in the children

Infection in utero resulted in abortion in eight and stillborns in three cases (Table 2) L m was isolated from various organs

The infected children born alive were divided into Monnet s (20) three groups according to their age at the onset of neonatal listeriosis 22 cases with Early disease (≤2 days) four cases with Intermediate disease (3–5 days) and eight cases with Late disease (≥6 days)

Early disease (22 cases) Septicemia dominated this group (Table 3) Two children had meningo encephalitis but this was probably only part of a septicemia L m was isolated from various organs Median values of birth weight and gestational age indicated that the pregnancies had not gone on to full term The mortality was high (13 cases)

Intermediate disease (4 cases) Two of the patients had septicemia and two had meningo encephalitis (Table 3) L m was isolated from various organs In the two fatal septicemic cases birth weight was lower than that of the two who survived from meningo encephalitis

Late disease (8 cases) All had meningo encephalitis with growth of L m in the CSF

Table 3 *Neonatal listeriosis in Sweden 1958–1974 34 cases*

	Early disease (≤[illegible] days)	Intermediate disease (3–5 days)	Late disease (≥6 days)
Septicemia	19/2[illegible]	[illegible]/4	0/8
Meningitis	3/2	[illegible]/4	8/8
Mortality	13/[illegible]	[illegible]/4	1/8
Maternal fever	9/2	1/4	1/8
Discoloration of amniotic fluid	16/[illegible]1	1/3	1/5
L m pos culture in mother	10/14	1/	0/4
L m pos culture in child	[illegible]	4/4	8/8
Birth weight g (median)	[illegible] 440		3 460
Gestational age weeks (median)	37		38
Pos serology for listeria	15/16	1/3	0/3
Complicated parturition	8/ 2	0/4	1/8

O-aggl titer >1/40 and or CF titer >1/10

(Table 3) The median birth weight was significantly higher in this group than in the group of Early disease (Rank sum test $p<0\ 05$) The mortality was low

Most of the children with septicemia were in a bad condition already at birth Nineteen had signs of respiratory distress and circulatory failure Seventeen children had neurological symptoms mostly flaccidity or increased tone with twitching Seizures occurred in eight of these children seven of whom died All four children with petechiae died Vomiting and/or diarrhoea occurred in four children and enlargement of the liver in six Nine out of 17 children studied got fever and eight children including five with a birth weight of less than 2 500 g had a low or normal temperature

In the cases of meningo-encephalitis the symptoms were typical of purulent meningitis with fever *irritability* whimpering and sometimes loss of appetite A tense fontanelle was noted in six cases Six children had seizures which was fatal in one Four children had respiratory symptoms five had vomiting and/or diarrhoea and five children had enlargement of the liver All children except one weighing less than 2 500 g had fever

In the primary CSF the number of polymorphonuclear as well as of mononuclear cells was increased (Table 4) Polymorphonuclear cells dominated in eight children and mononuclear cells in five Protein in the CSF was substantially increased (Table 4) and the glucose concentration was decreased in only three out of five cases studied

Antimicrobial treatment Since these patients had been treated in different hospitals there was no uniformity of the chemotherapeutic treatment for which reason caution should be exercised in the evaluation of the

Table 4 *Findings in primary CSF in meningo encephalitis in neonatal listeriosis in Sweden 1958–1974 13 cases*

	No of cases	Median value	Range
Total number of cells per l	13	$3\ 630\times10^6$	$198–6\ 600\times10^6$
Polymorphonuclear per l	11	$1\ 963\times10^6$	[illegible]3–$3\ 400\times10^6$
Mononuclear per l	11	$1\ 000\times10^6$	[illegible]63–3 76[illegible]×10
Protein/g per l	10	2 70	1 [illegible]9–10 74
Polymorphonuclears in relation to mononuclears	13	Poly > Mono 8	Mono > Poly 5

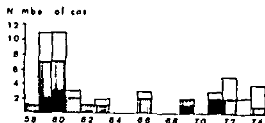


Fig 1 Annual incidence and outcome of neonatal listeriosis in Sweden 1958–1974 46 women (2 pairs of twins) ■ = abortions □ = livebirths (survivors) ▨ = stillborns ▥ = livebirths (succumbed later)

Table 2 *Abortions and stillborns in listeriosis in Sweden 1958–1974 11 cases*

Abortion	8/11
Stillborn	3/11
Maternal fever	5/11
Discoloration of amniotic fluid	?/3
L m pos culture in mother	8/9
L m pos culture in foetus	8/8
Serology positive	6/8

O aggl titer >1/40 and/or CF titer >1/10

Clinical information was obtained from a study of all patients records and was sometimes supplemented by direct information from the patients and their relatives or from members of the hospital staff

Part of this material has been published earlier as case reports (? 6 8 9 10 12 14 19 21 22 26 27) Seasonal variation geographic distribution animal contacts sero types and other epidemiological aspects are reported elsewhere (15) The pathological changes found at *necropsy* are published separately (18)

Bacteriology The technique used for bacteriological culture and diagnosis of Listeria monocytogenes has been described elsewhere (15)

Serology Antibodies against L m were assayed according to Winblad (15 28)

RESULTS

After the discovery of the first case in 1958 there were rather many cases in 1959 and 1960 Since then the frequency has been rather even and low (Fig 1)

Clinical manifestation

Eight pregnancies ended in abortion and three in stillborn foetuses (Table 1) Thirty seven children were born alive and 21 of these had septicemia a name we prefer to the earlier designation Granulomatosis infantiseptica

Table 1 *Listeriosis during pregnancy in Sweden 1958–1974*

Clinical diagnosis 46 women (two pairs of twins)

Abortion	8
Stillborn	3
Children born alive	
Granulomatosis infantiseptica	21 (15 fatal)
Meningo-encephalitis	13 (1 fatal)
No listeria infection	3 (1 fatal)

which proved fatal in 15 Thirteen children had meningo-encephalitis which was fatal in one Three of the infected women delivered children with apparently no clinical or bacteriological signs of listeriosis One of these children was born in the 24th week of pregnancy and soon died because of its immaturity

Clinical course in the children

Infection in utero resulted in abortion in eight and stillborns in three cases (Table 2) L m was isolated from various organs

The infected children born alive were divided into Monnet s (20) three groups according to their age at the onset of neonatal listeriosis 22 cases with Early disease (≤2 days) four cases with Intermediate disease (3–5 days) and eight cases with Late disease (≥6 days)

Early disease (22 cases) Septicemia dominated this group (Table 3) Two children had meningo encephalitis but this was probably only part of a septicemia L m was isolated from various organs Median values of birth weight and gestational age indicated that the pregnancies had not gone on to full term The mortality was high (13 cases)

Intermediate disease (4 cases) Two of the patients had septicemia and two had meningo encephalitis (Table 3) L m was isolated from various organs In the two fatal septicemic cases birth weight was lower than that of the two who survived from meningo encephalitis

Late disease (8 cases) All had meningo encephalitis with growth of L m in the CSF

Table 5 *Comparison between distribution of maximal antibody titers in two materials of registered blood donors and three materials of listeriosis*

Category	Total	O aggl pos (>1 40) (%)	CF pos (>1 10) (%)	O aggl pos CF pos (%)
Blood donors 1971–1975	53	8	4	1 6
Blood donors 1977	100	9	4	2
Women with listeriosis during pregnancy	?7	78	63	59
Mothers of children with late type of listeriosis	4	0	0	0
Adults and juveniles with listerial meningitis and septicemia	35	49	40	6

much less common One woman was delivered by Caesarian section

The placentae Eighteen women had pathological changes in the placenta Another 26 placentae were examined macroscopically only and found to be normal All seven placentae analysed in cases of abortion and stillbirth had pathological changes and all but one had signs of placentitis In the group of Early disease four women had pathological changes with infarctions clots and/or calcifications and another three women showed signs of placentitis One woman in the group of Late disease had pathological changes with calcifications and duplex placenta

Serotype of L m The distribution of serotypes 1 and 4b was fairly uniform (Table 6) A few strains were diagnosed as serotype 2 and 3 Epidemiological aspects of the serotypes are discussed in an earlier paper (15)

Antimicrobial treatment Since the women were treated in different hospitals the chemotherapy of listeriosis was not uniform Caution must therefore be exercised in the evaluation of the effect of the treatment Two women were treated during pregnancy One woman with SLE developed septicemia during pregnancy and was treated with benzyl penicillin 10 MU a day parenterally She had an abortion after eight days of therapy The other woman was treated with a sulphonamide because of symptoms of urinary tract infection After four days blood culture yielded growth of L m and ampicillin parenterally was given

Table 6 *Distribution of serotypes in relation to clinical diagnosis in Sweden 1958–1974*

Serotyping according to Winblad Early disease consisted of 19 gran infantisept and 3 men encephalit Intermediate disease consisted of gran infantisept and ? men encephalit Late disease consisted of 8 men encephalit

	Serotype					
	1		3	4b	Unknown	Total
Abortion	4	1		1	2	8
Stillborn	1				^	3
Early disease (≤ days)	9	?	1	8	2	2
Intermed disease (3–5 days)	1			1	?	4
Late disease (≥6 days)	5	1		2		8
No illness				3		3
	0	4	1	15	8	48*

Nine cases were serotyped by Seeliger Wurzburg Germany
* 46 women with two pairs of twins

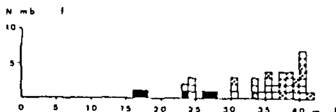


Fig 2 Gestational age in listeriosis in Sweden 1958–1974 41 neonates and foetuses ■ = abortions ▨ = still borns ⊠ = live births with men-encephalitis □ = live births with septicemia □ = live births with no listeria infection

effect of chemotherapy Sulphonamide combined with other drugs was successful in four cases It failed in one case Tetracycline combined with other drugs was successfully used in all five cases in the beginning of the period covered by the material Ampicillin combined with gentamicin in four cases and ampicillin combined with other drugs in three cases was successful In two cases this treatment failed In 17 surviving cases the therapy was continued for a median period of 20 days (range 8–41 days)

Outcome Most of the deaths in the group Early disease occurred within two days A girl with meningo encephalitis got hydrocephalus and died later (at 65 days) from seizures and respiratory arrest

Twenty children survived the neonatal listeriosis *Two children got hydrocephalus* and serious mental retardation one child had a slight spastic diplegia and one developed convergent strabismus

A mongoloid girl recovered from septicemia but died at three months from gastro enteritis Fifteen children are apparently healthy with normal psychomotor development The surviving children were followed up by pediatric specialists during a median period of 20 months Fourteen children were followed up for at least one year

Clinical course in the mothers

The median age of the mothers was 27 years (range 17–39 years) Twenty five of them had been pregnant before

Forty three women were apparently healthy One woman had systemic lupus erythematosus (SLE) another woman was treated during pregnancy because of thyrotoxicosis and one woman had rheumatoid arthritis None of the women were treated with corticosteroids or cytostatics

Laboratory findings A few women (10 cases) tested showed moderate leukocytosis (median value 12 0×10⁹ range 4 3–28 4×10⁹) All these women had growth of L m Of nine *women studied five had neutrophilic granulocytosis* and none had monocytosis The erythrocyte sedimentation rate (ESR) was raised *(median 69 mm range 20–113)*

Gestational age All the cases of listeriosis occurred after the 16th week of pregnancy (Fig 2)

Serology Serological analysis was done in 31 cases (Table 5) Listerial infection in pregnancy resulted in raised antibody titers significantly more often than after meningitis and septicemia in adults and juveniles ($p<0.05$) Of 11 women studied within six days after delivery ten had already developed positive titers

Seventeen women had apparently had normal pregnancies and four out of nine studied had growth of L m in the genital tract Another 17 women showed signs of infection and *11 out of 13 examined had L m in the genital* tract Usually the women had chills and fever a few days before delivery combined with muscle pain headache and tiredness Five women showed signs of urinary tract infection After delivery they recovered and became afebrile most of them rather soon without or in association with the beginning of antibiotic treatment Other complications like bleeding hypertension with edema and albuminuria and premature contractions occurred in another 12 women In 21 the amniotic fluid was discolored

The mothers of children with Early disease often showed signs of infection (Table 3) In eight of these women parturition was complicated In the mothers of the children with Late disease signs of infection were

nancy terminated in foetal death Unfortunately neither necropsy nor culture was done in these two cases

DISCUSSION

The present investigation confirmed and extended earlier observations (20) that neonatal listeriosis occurs in two distinct forms which differ in time of onset mode of transmission clinical symptoms and prognosis

In this study 22 infants fell ill within the first two days of life Typically they had septicemia with nonspecific signs of respiratory distress and circulatory failure that were evident already at birth (1 3 24) Seizures and petechiae indicated a bad prognosis In these cases the infection must have been transmitted during intrauterine life The amniotic fluid was nearly always discolored and the mothers had typical fever growth of L m in the genital tract and positive serology Most of the infants were born prematurely and had low birth weights They were in a bad condition and the mortality was high most of them dying within two days

In contrast all eight infants with Late disease had meningo encephalitis They were born at expected time and had normal birth weights When they fell ill after five days of life they developed the usual signs of purulent meningitis with fever Mononuclears often dominated the cells in the primary CSF suggesting virus or other agents The definite diagnosis however should be based on isolation of the microorganism (13 24) Their mothers were healthy The amniotic fluid was normal In no case was L m cultured from the genital tract Serology remained negative Thus the disease was evidently transmitted from the environment In other studies nosocomial outbreaks of Late disease have been reported (15 16) The prognosis was good and all but one survived

In no case infection during delivery was suspected but since culture of the genital tract was seldom performed in Late disease the material did not exclude this possibility

Infants falling ill when 3–5 days old belonged to an intermediate group of overlapping cases Two were clinically of early type and two were clinically of late type

Stillborns and abortions showed signs of intrauterine infection and resembled Early disease

As in other manifestations of listeriosis (3 15 17 24) serotypes 1 and 4 b dominated and were not correlated with the type of infection or the severity of the disease

An earlier calculated perinatal mortality of about 1% (14) was not confirmed As reported elsewhere (15) our material showed that listeriosis was diagnosed annually in up to 1 per thousand of all stillborns and infants who died within the first week of life (perinatal mortality) Of the total neonatal mortality in Sweden listeriosis was the cause of up to 3 7 per thousand of all fatal cases within the first 27 days of life The results resemble those of Breuning & Fritzsche (4) who found a death rate of 0 154% due to listeriosis

Among the surviving children four had sequelae after the infection Out of 48 pregnancies 15 children are still living and apparently healthy This indicates the severity of listeriosis If the physician suspects listeriosis antibiotic treatment should be started immediately

The effect of chemotherapy in the children was difficult to evaluate Ampicillin was effective in some cases and is to be regarded as the main drug in the treatment of listeriosis in newborns (25) In vitro studies suggest that combined ampicillin and gentamicin should be used (7) Erythromycin is an alternative Seeliger (25) recommended continuation of treatment for up to 21 days

Co existing disorders in the mothers were not common but did occur in a few women The higher frequency of raised antibody titers in pregnant women may have been due to the fact that most of them were previously healthy while adult patients with other manifestations of listeriosis were often immunocompromised

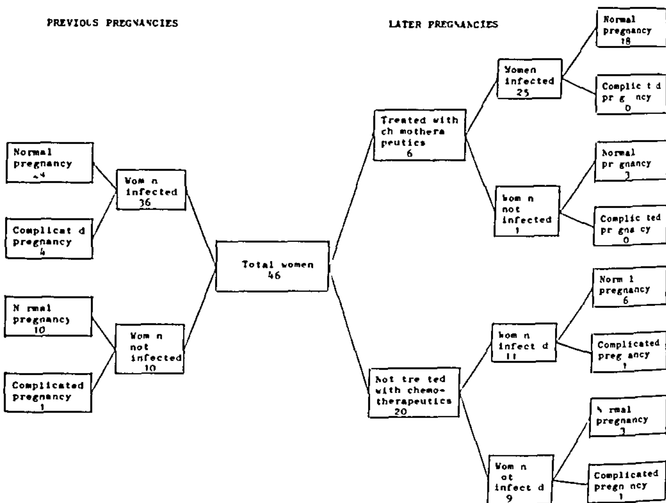


Fig 3 Previous and later pregnancies in 46 women with listeriosis in Sweden 1958–1974 Women with septicemia abortion stillbirth or mothers of children with Early disease or early type of Intermediate disease were classified as women infected Mothers of children with Late disease or late type of Intermediate disease were classified as women not infected

instead of sulphonamide By then she had already become afebrile Two days later she gave birth to a healthy boy

In 20 women no chemotherapy of listeriosis was given after delivery In another 24 women such treatment was given after delivery Ampicillin (7 patients) penicillin (7 patients) and tetracycline (5 patients) were most commonly used alone or combined with other drugs The duration of treatment ranged from four to 32 days (median 10 days)

Previous and later pregnancies Before the pregnancy with listeriosis 34 pregnancies were normal and five were complicated including four abortions and one case of vacuum extraction because of asphyxia (Fig 3) One woman had two abortions One of these foetuses was examined at necropsy and showed no signs of infection There was no statistical significant difference in the frequency of complicated pregnancies in women who were later apparently infected with L m than in women who were not infected

Twenty six women treated with chemotherapeutics later had altogether 21 pregnancies without complications In the group of 20 women not treated with chemotherapeutics there were later nine normal pregnancies and two complicated ones (Fig 3) One woman got a stillborn The death was caused by listeriosis Later on she had an abortion Another woman had a child with the Late disease from neonatal listeriosis probably a case of nosocomial infection A later preg

nancy terminated in foetal death Unfortunately neither necropsy nor culture was done in these two cases

DISCUSSION

The present investigation confirmed and extended earlier observations (20) that neonatal listeriosis occurs in two distinct forms which differ in time of onset mode of transmission clinical symptoms and prognosis

In this study 22 infants fell ill within the first two days of life Typically they had septicemia with nonspecific signs of respiratory distress and circulatory failure that were evident already at birth (1 3 24) Seizures and petechiae indicated a bad prognosis In these cases the infection must have been transmitted during intrauterine life The amniotic fluid was nearly always discolored and the mothers had typical fever growth of L m in the genital tract and positive serology Most of the infants were born prematurely and had low birth weights They were in a bad condition and the mortality was high most of them dying within two days

In contrast all eight infants with Late disease had meningo encephalitis They were born at expected time and had normal birth weights When they fell ill after five days of life they developed the usual signs of purulent meningitis with fever Mononuclears often dominated the cells in the primary CSF suggesting virus or other agents The definite diagnosis however should be based on isolation of the microorganism (13 24) Their mothers were healthy The amniotic fluid was normal In no case was L m cultured from the genital tract Serology remained negative Thus the disease was evidently transmitted from the environment In other studies nosocomial outbreaks of Late disease have been reported (15 16) The prognosis was good and all but one survived

In no case infection during delivery was suspected but since culture of the genital tract was seldom performed in Late disease the material did not exclude this possibility

Infants falling ill when 3–5 days old belonged to an intermediate group of overlapping cases Two were clinically of early type and two were clinically of late type

Stillborns and abortions showed signs of intrauterine infection and resembled Early disease

As in other manifestations of listeriosis (3 15 17 24) serotypes 1 and 4 b dominated and were not correlated with the type of infection or the severity of the disease

An earlier calculated perinatal mortality of about 1% (14) was not confirmed As reported elsewhere (15) our material showed that listeriosis was diagnosed annually in up to 1 per thousand of all stillborns and infants who died within the first week of life (perinatal mortality) Of the total neonatal mortality in Sweden listeriosis was the cause of up to 3 7 per thousand of all fatal cases within the first 27 days of life The results resemble those of Breuning & Fritzsche (4) who found a death rate of 0 154% due to listeriosis

Among the surviving children four had sequelae after the infection Out of 48 pregnancies 15 children are still living and apparently healthy This indicates the severity of listeriosis If the physician suspects listeriosis antibiotic treatment should be started immediately

The effect of chemotherapy in the children was difficult to evaluate Ampicillin was effective in some cases and is to be regarded as the main drug in the treatment of listeriosis in newborns (25) In vitro studies suggest that combined ampicillin and gentamicin should be used (7) Erythromycin is an alternative Seeliger (25) recommended continuation of treatment for up to 21 days

Co-existing disorders in the mothers were not common but did occur in a few women The higher frequency of raised antibody titers in pregnant women may have been due to the fact that most of them were previously healthy while adult patients with other manifestations of listeriosis were often immunocompromised

There was no early abortion in our material and this is only rarely described (3 24)

Two women were treated with antibiotics during pregnancy In one of them treatment with benzyl penicillin could not prevent the infection of the foetus resulting in abortion The woman however suffered from SLE with depressed cellular immunity which is most important for the resistance to listeriosis (5 11) The other woman was treated with a sulphonamide and later with ampicillin and delivered a healthy boy If listeriosis is diagnosed in a pregnant woman ampicillin should be used Erythromycin is an alternative

Many authors recommend antibiotic treatment of the mother when listeriosis is diagnosed after the delivery The present experience did not indicate any advantage of such treatment (Fig 3) Only two pregnancies in women who were not treated resulted in abortion and stillbirth respectively and in neither case was it proved that the fatal issue was due to listeriosis However Rappaport et al (23) and other authors have reported cases of women with repeated abortions in their opinion caused by a persistent infection of the genital tract with L m Bojsen Moller (3) was not convinced and stated that so far no definite case has been reported in which L m has been isolated twice in the same patient in two successive abortions or births No prospective investigation of this question is on record For the time being treatment of a mother with clinical or bacteriological signs of infection is recommended Ampicillin is to be preferred In patients allergic to penicillin tetracycline erythromycin or co trimoxazole are the alternatives Seeliger (25) recommends oral treatment for 8–10 days

REFERENCES

1 Becroft D M O Farmer K Seldon R J Sowden R Stewart J H Vines A & Wattie D A Epidemic listeriosis in the newborn *Br Med J III* 747 1971

2 Bergman P Kjellander J Thunell G & Zetterberg L Listerios och graviditet *Lakartidningen 57* 24 1960

3 Bojsen Moller J Human listeriosis Diagnostic epidemiological and clinical studies *Acta Pathol Microbiol Scand (B)* Suppl 229 1972

4 Breuning M & Fritzsche F Uber die Haufigkeit der Listeriose bei Neugeborenen *Geburtshilfe Frauenheilkd 14* 1113 1954

5 Buchner L H & Schneiersson S S Reviews Clinical and laboratory aspects of Listeria monocytogenes infections With a report of ten cases *Am J Med 45* 904 1968

6 Ekelund H Laurell G Melander S Olding L & Vallqvist B Listeria infection in the foetus and the newborn *Acta Paediatr Scand 51* 698 1962

7 Espaze E P Auduner A & Courtieu A L Action in vitro of some antibiotic combinations on Listeria monocytogenes *Proceedings of the VIIth International Symposium on the Problems of Listeriosis* Varna Bulgaria 1977 In press

8 Gullberg S Jernelius H & Kahlmeter O Tre fall av listeriameningit *Lakartidningen 57* 2117 1960

9 Hagberg B Olding L & Philipsson L Two cases of listeria infection in the newborn *Acta Paediatr Scand 48* 631 1959

10 Hedberg K Magnusson P H & Naglo O Ett par fall av listeros *Opuscula Medica 10* 365 1959

11 Ivarson S Lidin Janson G & Svensson R Listeric meningitis in the non-compromised host *Infection 5* 204 1977

12 Kayser K & Malmostrom-Groth A G Listeros—ytterligare fall hos spadbarn *Lakartidningen 57* 792 1960

13 Kromann Andersen C Human listeriose En oversigt og fire tilfælde *Ugeskr Laeger 132* 1310 1970

14 Larsson J Forekomsten av listeriainfektion i Sverige *Lakartidningen 57* 37 1960

15 Larsson S Epidemiology of listeriosis in Sweden 1958–1974 Accepted for publication in *Scand J Infect Dis*

16 Larsson S Cederberg Å Ivarsson S Svanberg L & Cronberg S Listeria monocytogenes causing hospital acquired entero colitis and meningitis in newborn infants *Br Med J II* 473 1978

17 Larsson S Cronberg S & Winblad S Clinical aspects on 64 cases of juvenile and adult listeriosis in Sweden *Acta Med Scand 204* 503 1978

18 Larsson S & Linell F Correlations between clinical and post mortem findings in listeriosis Accepted for publication in *Scand J Infect Dis*

19 Linell F Schiller S & Winblad S Listeriainfektion hos manniska *Nord Med 61* 181 1959

20 Monnet P Listeriosis in a neonatal unit *In* M Woodbine (ed) *Problems of listeriosis* Leicester University Press Sutton Bonington 1975 p 188

21 Olding L & Philipsson L Two cases of listeriosis in the newborn associated with placental infection *Acta Pathol Microbiol Scand 48* 24 1960

22 Palmén K & Sommerschild H Perinatal listeria meningit *Lakartidningen 61* 1781 1964

23 Rappaport F M Rabinovitz R T & Krochik N Genital listeriosis as a cause of repeated abortion *Lancet I* 1273 1960

24 Seeliger H P R *Listeriosis* S Karger A G Basel 1961

25 Seeliger H P R & Matheis H Review Treatment of human listeriosis *Ger Med Mon 14* 566 1969
26 Stormby K Wallengren O & Weiber A Två listeriosfall i nyfoddhetsperioden med troligt nosokomialt smittosamband *Lakartidningen 57* 21 2 1960
27 Soderling L Listeriosis i lundaregionen 1971–1972 *Lakartidn ngen 71* 2878 1974
28 Winblad S Studies in antibodies in human listeriosis I Antibodies in cases of bacteriologically verified human listeriosis *Acta Pathol Microbiol Scand 58* 1 3 1963

Submitted March 10 1978
Accepted Nov 28 1978

(S L) Department of Infectious Diseases
Malmo General Hospital
S 1401 Malmo
Sweden

Table 1 *Occurrence of precipitins against E coli B fragilis and P aeruginosa in sera from normal persons*

	Number of normal persons	Prevalence of persons with		
		0 precipitins	1 precipitin	2 precipitins
E coli	44	32 (73%)	9 (20%)	3 (7%)
B fragilis	44	37 (84%)	7 (16%)	
P aeruginosa	154	145 (94%)	9 (6%)	

From (10)

study These patients are examined every month as out patients at the Paediatric Clinic TG The diagnostic criteria for CF have been reported previously (5) The *monthly examination included bacteriological* examination of sputum or secretion obtained by endolaryngeal aspiration (6) Anaerobic culture of these respiratory secretions *was not attempted* since this is *meaningless if* percutaneous transtracheal aspiration is not employed in view of the abundance of anaerobes present in the normal flora of the upper respiratory tract (3) However microscopical examination of the secretions provided no evidence of anaerobes as being important pathogens in the respiratory tract of our CF patients Sera were obtained by venipuncture and stored at −30 C with 15 mM NaN_3/l added The sera from CF patients who had previously harboured *E coli* in the respiratory tract were collected on an average of 6 months (range 0–36 months) after the latest isolation of these bacteria Twenty one of the CF patients have succumbed but serum from *these patients was available from a large collection of* specimens stored as described These sera were collected on an average 5½ months before death (range 1 day–18 months)

Control persons

Sera from 154 normal persons covering all age groups comprise the control group concerning *P aeruginosa* precipitins (10) and sera from 44 normal persons (19 males 25 females) the control group as regards precipitins against *E coli* and *B fragilis* The mean age of these 44 persons was 20 years (range 6–59 years) and 30 of them were younger than 30 years

Precipitating antibodies against P aeruginosa E coli and B fragilis

Crossed immunoelectrophoresis (microtechinque (7 21 22)) was employed to examine serum from the patients for precipitating antibodies against antigens obtained by sonication of the three bacterial species (5 9) The *P aeruginosa* antigen sample contains at least 64 different antigens (5 11) Colloid concentration is 11 8 g/l (refractometry human IgG as standard)

The *E coli* (021 H27) antigen *sample contains* at least 42 antigens visualized as 42 immunoprecipitates with a corresponding rabbit antiserum in crossed immunoelectrophoresis *colloid concentration* 14 8 g/l and 37 of the 42 antigens are common *E coli* antigens (9)

The *B fragilis ss thetaiotaomicron* (VPI 5) antigen sample contains at least 34 antigens visualized as 34 immunoprecipitates when run against a corresponding rabbit antiserum in crossed immunoelectrophoresis colloid concentration 5 8 g/l and 19 of the 34 antigens are common antigens of other *B fragilis* subspecies (9) *Only one* of the *P aeruginosa* antigens cross reacts with *B fragilis ss thetaiotaomicron* and five of the *P aeruginosa* antigens and one *B fragilis ss thetaiotaomicron* antigen cross react with *E coli* (9)

By means of crossed immunoelectrophoresis the antibody response against *P aeruginosa* is quantified by the number of precipitins (= the number of immunoprecipitates) which is correlated with the titres of the precipitins (11) and the same approach is used to quantify the antibody response against *E coli* and *B fragilis*

Statistical methods

The χ Square test and the Mann Whitney test (? 17) were used Level of significance 5% (double tailed tests)

RESULTS

A significantly higher prevalence of precipitins against *E coli* was found in CF sera (76%) as compared with the normal sera (27% $p<0.0005$) and 38% of the CF sera contained ≥ 3 *E coli* precipitins (Fig 1 and Table 1) The mean number of precipitins in positive

Table 2 *Occurrence of precipitins against E coli in sera from cystic fibrosis patients who have harboured and those who have not harboured E coli in the lungs*

	CF without *E coli* [a]	CF with *E coli* [b]	Total
0–2 precipitins	55 (67%)	27 (33%)	82
≥ 3 precipitins	37 (73%)	14 (27%)	51
Total	92 (69%)	41 (31%)	133
		n s	

[a] Average observation time 4 9 years
[b] Average observation time 3 3 years

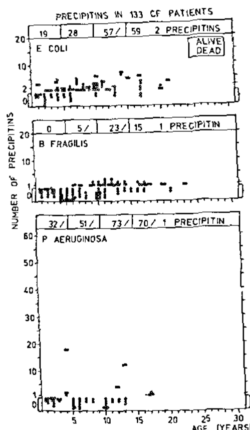


Fig 1 Number of precipitins against *E coli* *B fragilis* and *P aeruginosa* in serum of 133 cystic fibrosis patients plotted against the age of the patients ○ Patients still alive ● patients who subsequently succumbed The highest number of precipitins against these bacteria revealed in sera from normal persons is indicated by the broken lines As regards these lines the prevalences of increased numbers of precipitins against the bacteria in the different age groups are given at the top of each diagram (*E coli* > precipitins *B fragilis* >1 precipitin *P aeruginosa* >1 precipitin)

sera was 3 (range 1–12) and the prevalence of precipitins increased significantly with age ($p<0.005$) (Fig 1) The antibody response against *E coli* was not correlated with previous colonization of the respiratory tract by these bacteria (Table 2) nor with the prognosis (Fig 1)

A significantly higher prevalence of precipitins against *B fragilis* was found in CF sera (38%) as compared with the normal sera ($p<0.01$) [illegible] CF sera contained ≥ 2 *B fragilis* precipitins (Fig 1 Table 1) The mean number of precipitins in positive sera was 1 4 (range 1–4) and the prevalence of precipitins increased significantly with age ($p<0.0005$) (Fig 1) The antibody response against *B fragilis* was not correlated with the prognosis (Fig 1)

A significantly higher prevalence of precipitins against *P aeruginosa* was found in CF sera (63%) as compared with normal sera ($p<0.0005$) and 55% of the CF sera contained ≥ 2 *P aeruginosa* precipitins (Fig 1 Table 1) The mean number of precipitins in positive sera was 16 (range 1–60) The prevalence of precipitins increased significantly with age ($p<0.0005$) The prevalence of ≥ 2 precipitins was significantly higher in the CF patients who had harboured these bacteria in the respiratory tract as compared with those who had never done so ($p<0.0005$) (Table 3) The number of precipitins was significantly higher in these chronically infected patients (mean 22) as compared with the CF patients with only intermittent *P aeruginosa* colonization (mean 2 $p<0.0001$) The number of *P aeruginosa* precipitins was significantly higher in CF patients who have subsequently succumbed (mean 27 precipitins) compared with the patients who are still alive (mean 12 precipitins $p<0.0005$) (Fig 1)

The prevalence and number of precipitins in CF patients was significantly higher against *P aeruginosa* than against *E coli* and *B fragilis* ($p<0.001$) (Fig 1) Tables 4 and 5 show that the presence of increased numbers of *E coli* and *B fragilis* precipitins was associated significantly with increased numbers of *P aeruginosa* precipitins

DISCUSSION

The antibody response against *P aeruginosa* was much more pronounced than that against *E coli* and *B fragilis* Besides being correlated with chronic *P aeruginosa* lung infection and poor prognosis the antibody response against *P aeruginosa* has also been shown to be cor

Table 3 *Occurrence of precipitins against P aeruginosa in relation to the intermittent or chronic presence of P aeruginosa in the lungs of cystic fibrosis patients (6 11)*

	CF without *P aeruginosa*	CF with *P aeruginosa* intermittently	CF with *P aeruginosa* chronically	Total
0–1 precipitin	35 (58%)	25 (42%)		60
≥ 2 precipitins	1 (1 5%)	14 (19%)	58 (79 5%)	73
Total	36 (27%)	39 (29%)	58 (44%)	133
			$p<0\ 0005$	

related with inflammation and tissue damage the presence of immune complexes in serum and especially in sputum and poor lung function (7 11 14) In contrast the antibody response against *E coli* and *B fragilis* was not correlated with respiratory infections nor with the prognosis of the CF patients and infections outside the respiratory tract have not been observed in our patients (6 11) Accordingly the question is whether antibodies against *E coli* and *B fragilis* may be induced without the presence of infections caused by these bacteria

Increased antibody response against these bacteria was found predominantly in CF patients with increased antibody response against *P aeruginosa* Several explanations for this interesting association may be offered (*i*) Cross reactive *P aeruginosa* antigens may account for at least some of the antibody response against *E coli* in some of the patients This is less probable in view of the antibody response against *B fragilis* which only cross reacts with one of the *P aeruginosa* antigens as compared with *E coli* which cross reacts with five of the *P aeruginosa* antigens (9) (*ii*) The presence of some *P aeruginosa* antigens e g the lipopolysaccharide might induce an adjuvant effect on the antibody response to other antigens (13 16) However as immunosuppressive effects of *P aeruginosa* have also been reported (15) this explanation is doubtful (*iii*) In accordance with the hypothesis of Wallwork et al (19) it is possible that the antibody response against *E coli* and *B fragilis* is induced by antigens from these species which have been absorbed from the gut

A generalized increased absorption of antigens from mucosal surfaces due to a transient defect of secretory IgA seems less likely in view of the relatively low prevalence of antibodies against the abundant intestinal flora (*E coli* 10^{8}–10^{9} and *B fragilis* 10^{11}/g dry weight of faeces (3)) as compared with the high prevalence of antibodies against the res

Table 4 *Relationship between occurrence of precipitins against P aeruginosa and E coli in sera from cystic fibrosis patients*

	0–2 *E coli* precipitins	≥ 3 *E coli* precipitins
0–1 *P aeruginosa* precipitin	50 (37 5%)	10 (7 5%)
≥ 2 *P aeruginosa* precipitins	32 (24%)	41 (31%)
		$p<0\ 0005$

Table 5 *Relationship between occurrence of precipitins against P aeruginosa and B fragilis in sera from cystic fibrosis patients*

	0–1 *B fragilis* precipitin	≥2 *B fragilis* precipitins
0–1 *P aeruginosa* precipitin	60 (45%)	1 (1%)
≥2 *P aeruginosa* precipitins	60 (45%)	12 (9%)
		$p<0\ 005$

piratory pathogen *P aeruginosa* although the systemic antibody response against anti gens absorbed from the intestinal tract to the venous blood is possibly less pronounced due to filtration by the liver (1)

However the malabsorption theory (19) may be modified to explain the results of the present work Antigens from the gut flora which cross react with *P aeruginosa* antigens must be present in the gut of all CF patients (9) Furthermore *P aeruginosa* antigens must be present in the gut of the patients who har bour these bacteria in the respiratory tract due to sputum being swallowed The presence of such antigens in the gut provides the possi bility of immune reactions in the gut mucosa between these antigens in the gut and *P aeru ginosa* antibodies originating from serum in patients with chronic *P aeruginosa* lung infec tion Such immune reactions have been shown to increase absorption of other antigens through mucosal membranes *in vitro* (18) and evidence of immune complexes in biopsies from the gut of succumbed CF patients has actually been reported (12) A transient secre tory IgA defect (20) would probably augment the possibility of such reactions

In conclusion evidence has been presented which points to increased absorption of anti gens from the gut of CF patients It is sug gested that the increased absorption is sec ondary to immune reactions in the intestinal mucosa

ACKNOWLEDGEMENTS

This work was supported by grants from the Family Hede Nielsen s Foundation and the Danish Medical Research Council Thanks are due to the technical staff of the Department of Clinical Microbiology Hvidovre Hospital for skilful technical assistance

REFERENCES

1 Bjørneboe M Prytz H & Ørskov F Antibodies to intestinal microbes in serum of patients with cir rhosis of the liver *Lancet i* 58 197
2 Diem K (ed) *Documenta Geigy Scientific Tables* J A Geigy S A Basel 196 pp 36 174
3 Finegold S M *Anaerobic Bacteria in Human Disease* Academic Press New York 1977 pp 26 41 223
4 Gibbons A Allan J D Holzel A & McFarlane H Cell mediated immunity in patients with cystic fibro is *Br Med J i* 120 1976
5 Høiby N & Axelsen N H Identification and quantitation of precipitins against Pseudomonas aeruginosa in patients with cystic fibrosis by means of crossed immunoelectrophoresis with intermediate gel *Acta Pathol Microbiol Scand (B) 81* 298 1973
6 Høiby N Epidemiological investigations of the res piratory tract bacteriology in patients with cystic fibrosis *Acta Pathol Microbiol Scand (B) 82* 541 1974
7 Høiby N Pseudomonas aeruginosa infection in cystic fibrosis Relationship between mucoid strains of Pseudomonas aeruginosa and the humoral immune response *Acta Pathol Microbiol Scand (B) 82* 551 1974
8 Høiby N & Wiik A Antibacterial precipitins and autoantibodies in serum of patients with cysti fi brosis *Scand J Respir Dis 56* 38 1975
9 Høiby N Cross reactions between Pseudomonas aeruginosa and thirty six other bacterial species *Scand J Immunol 4* (Suppl 2) 187 1975
10 Høiby N Antibodies against Pseudomonas aerugi nosa in serum from normal persons and patients colonized with mucoid or non mucoid Pseudomonas aeruginosa results obtained by crossed immunoelec trophoresis *Acta Pathol Microbiol Scand (C) 85* 142 1977
11 Høiby N Pseudomonas aeruginosa infection in cystic fibrosis Diagnostic and prognostic significance of Pseudomonas aeruginosa precipitins determined by means of crossed immunoelectrophoresis A survey *Acta Pathol Microbiol Scand (C)* Suppl *26* pp 3–96 1977
12 McFarlane H Holzel A Brenchley P Allan J D Wallwork J C Singer B E & Worsley B Immune complexes in cystic fibro is *Br Med J i* 4 3 1975
13 Sasaki M Ito M & Homma J Y Immunologi al studies on the original endotoxin protein (OEP) of Pseudomonas aeruginosa Adjuvant effect of OEP in vivo *Japan J Exp Med 45* 335 1975
14 Schiøtz P O Nielsen H Høiby N Glikmann G & Svehag S E Immune complexes in the sputum of patients with cystic fibrosis suffering from chronic Pseudomonas aeruginosa lung infection *Acta Pathol Microbiol Scand (C) 86* 37 1978
15 Schwab J H Suppression of the immune response by microorganisms *Bacteriol Rev 39* 121 1975
16 Skidmore B J Chiller J M Morrison D C & Weigle W O Immunologic properties of bacterial lipopolysaccharide (LPS) correlation between the mitogenic adjuvant and immunogenic activites *J Immunol 114* 770 1975
17 Therkelsen A J *Medicinsk Statistik* Akademisk Boghandel Århus 1968 pp 140 146
18 Tolo K Brandtzaeg P & Jonsen J Mucosal pene tration of antigen in the presence or absence of serum derived antibody *Immunol 33* 733 1977

Table 3 *Occurrence of precipitins against P aeruginosa in relation to the intermittent or chronic presence of P aeruginosa in the lungs of cystic fibrosis patients (6 11)*

	CF without *P aeruginosa*	CF with *P aeruginosa* intermittently	CF with *P aeruginosa* chronically	Total
0–1 precipitin	35 (58%)	25 (42%)		60
≥ 2 precipitins	1 (1 5%)	14 (19%)	58 (79 5%)	73
Total	36 (27%)	39 (29%)	58 (44%)	133
			p<0 0005	

related with inflammation and tissue damage the presence of immune complexes in serum and especially in sputum and poor lung function (7 11 14) In contrast the antibody response against *E coli* and *B fragilis* was not correlated with respiratory infections nor with the prognosis of the CF patients and infections outside the respiratory tract have not been observed in our patients (6 11) Accordingly the question is whether antibodies against *E coli* and *B fragilis* may be induced without the presence of infections caused by these bacteria

Increased antibody response against these bacteria was found predominantly in CF patients with increased antibody response against *P aeruginosa* Several explanations for this interesting association may be offered (*i*) Cross reactive *P aeruginosa* antigens may account for at least some of the antibody response against *E coli* in some of the patients This is less probable in view of the antibody response against *B fragilis* which only cross reacts with one of the *P aeruginosa* antigens as compared with *E coli* which cross reacts with five of the *P aeruginosa* antigens (9) (*ii*) The presence of some *P aeruginosa* antigens e g the lipopolysaccharide might induce an adjuvant effect on the antibody response to other antigens (13 16) However as immunosuppressive effects of *P aeruginosa* have also been reported (15) this explanation is doubtful (*iii*) In accordance with the hypothesis of Wallwork et al (19) it is possible that the antibody response against *E coli* and *B fragilis* is induced by antigens from these species which have been absorbed from the gut

A generalized increased absorption of antigens from mucosal surfaces due to a transient defect of secretory IgA seems less likely in view of the relatively low prevalence of antibodies against the abundant intestinal flora (*E coli* 10^{8}–10^{9} and *B fragilis* 10^{11}/g dry weight of faeces (3)) as compared with the high prevalence of antibodies against the res

Table 4 *Relationship between occurrence of precipitins against P aeruginosa and E coli in sera from cystic fibrosis patients*

	0–2 *E coli* precipitins	≥ 3 *E coli* precipitins
0–1 *P aeruginosa* precipitin	50 (37 5%)	10 (7 5%)
≥ 2 *P aeruginosa* precipitins	32 (24%)	41 (31%)
		p<0 0005

Table 5 *Relationship between occurrence of precipitins against P aeruginosa and B fragilis in sera from cystic fibrosis patients*

	0–1 *B fragilis* precipitin	≥2 *B fragilis* precipitins
0–1 *P aeruginosa* precipitin	60 (45%)	1 (1%)
≥2 *P aeruginosa* precipitins	60 (45%)	12 (9%)
		p<0 005

Acta Pædiatr Scand 68 501-506 1979

SUPPRESSED LYMPHOCYTE MITOGEN RESPONSIVENESS IN URINARY TRACT INFECTIONS OF CHILDREN AND ITS CORRELATION TO PYELONEPHRITIS

M JANAS T VESIKARI O HALLSTRÖM and R ANTTILA

From the Departments of Paediatrics and Microbiology Tampere Central Hospital and from the Institute of Clinical Sciences University of Tampere Tampere Finland

ABSTRACT Janas M Vesikari T Hallstrom O and Anttila R (Departments of Paediatrics and Microbiology Tampere Central Hospital and Institute of Clinical Sciences University of Tampere Tampere Finland) Suppressed lymphocyte mitogen responsiveness in urinary tract infections of children and its correlation to pyelonephritis Acta Paediatr Scand 68 501 1979 —Cell mediated immunity (CMI) was studied in a group of 48 children with urinary tract infections (UTI) using a whole blood micromethod for lymphocyte stimulation in vitro The patients were subdivided into pyelonephritis group (27 cases) and lower urinary tract infection (LUTI) group (21 cases) on the basis of fever erythrocyte sedimentation rate C reactive protein and renal concentration capacity At the acute stage of infection the lymphocyte responsiveness to leucoagglutinin (LA) and concanavalin A (Con A) was suppressed in both groups but the suppression was much greater in those with pyelonephritis By 6 weeks after infection the lymphocyte responses were normal in most but not all cases We conclude that an acute pyelonephritis is associated with marked suppression of CMI and that the latter can be used as an additional criterion for establishing the level of infection Patients with UTI did not generally appear to have any primary defect of CMI but when suppression of CMI was present it seemed secondary to an ongoing infection

KEY WORDS Cell mediated immunity lymphocyte stimulation urinary tract infections pyelonephritis

In the pathogenesis of urinary tract infections (UTI) in children bacterial interaction with uroepithelial cells and anatomical defects of the urinary tract are believed to play major roles (5 14) Humoral immunity in terms of serum immunoglobulin levels and specific antibody response to bacterial antigens seems generally intact (5 8)

Defective cell mediated immunity (CMI) could conceivably contribute to the pathogenesis of recurrent UTI either as a primary deficiency or having been temporarily suppressed by an external cause such as viral infection UTI itself particularly pyelonephritis may also suppress CMI (9 12) Both normal and impaired CMI have been reported in chronic and recurrent UTI in children but the various mechanisms have not been considered

We report lymphocyte stimulation in vitro as a measurement of CMI by the acute early convalescent and late convalescent stages of UTI in children Cases with pyelonephritis and lower urinary tract infection (LUTI) were analysed separately Since the immunosuppression appeared to bear correlation to the level of infection the lymphocyte stimulation test was considered as an additional parameter in the level diagnosis of UTI (in addition to fever C reactive protein (CRP) erythrocyte sedimentation rate (ESR) and renal concentration capacity)

MATERIALS AND METHODS

Patients

48 patients 45 girls and 3 boys presenting with acute UTI to the Department of Paediatrics Tampere Central Hospital were studied The diagnosis of UTI in most cases was based on significant bacterial growth in urine obtained by bladder aspiration Alternatively two clean voided

19 Wallwork J C Brenchley P McCarthy J Allen J D Moss D Ward A M Holzel A Williams R F & McFarlane H Some aspects of immunity in patients with cystic fibrosis *Clin Exp Immunol 18* 303 1974
20 Wallwork J C & McFarlane H The s IgA system and hypersensitivity in patients with cystic fibrosis *Clin Allergy* 6 349 1976
21 Weeke B General remarks on principles equipment reagents and procedures *Scand J Immunol* 2 (suppl 1) 15 1973
22 Weeke B Crossed immunoelectrophoresis *Scand J Immunol 2* (Suppl 1) 47 1973

Submitted Aug 31 1978
Accepted Jan 17 1979

(N H) Hvidovre Hospital
Klinisk Mikrobiologisk Afs 445
Kettegård Alle 30
DK 2650 Hvidovre
Denmark

Table 1 *Level diagnosis of UTI by standard criteria in 48 patients All patients in the pyelo nephritis group had fever 38 5 C or more*

Criteria	Positive/cases studied			
	Pyelonephritis (n=27)		*LUTI* (n=21)	
ESR ≥ 5 mm/h	21/ 1	100%	3/16	19%
CRP ≥10 μg/ml	18/21	86%	0/16	0%
Urine osmolality <800 mOsm/l	0/21	95%	4/11	25%

Lower urinary tract infection

(Sigma) Each mitogen was used at 7 different concen tractions The concentration of LA ranged from 50 μg/ml to 0 5 μm/ml and that of Con A from 500 μg to 5 μg/ml In addition for each test three cultures were set with no added mitogen

All the results are expressed as stimulation ratios (s r) obtained by dividing the counts in stimulated cultures (mean of three) by those in background cultures (mean of three) For statistical analysis of the results Student s *t* test was applied

CRP determinations

CRP concentrations were measured by a single radial im munodiffusion method (10) using LC Partigen CRP kit of Behring Institut

Renal concentrating capacity

The patients ability to concentrate urine was usually determined on the second day of hospitalization Two successive urine specimens were collected after 15 hours fasting The osmolality of the urine was measured by a freezing point osmometer The concentration capacity was considered reduced only if the osmolality of the urine in both samples was below 800 mOsm/l

Diagnostic criteria for pyelonephritis

The criteria for level diagnosis of UTI proposed by Jodal et al (6) were applied in a slightly modified form A case of UTI was classified as pyelonephritis if 3 of the follow ing 4 criteria were met Fever 38 5 C or more ESR 25 mm/hr or more CRP 10 μg/ml or more and renal con centrating capacity <800 mOsm/l (see Results)

According to these criteria 27 cases were classified as pyelonephritis Of the remaining 21 cases none was positive for more than one of the above criteria Regard less of the degree of symptoms all these cases were grouped as LUTI

RESULTS

Normal dose response curve for LA and Con A The best responsiveness of lymphocytes to LA was seen using the highest concentration of LA 50 μg/ml A still higher concentration 100 μg/ml did not improve the results but was not inhibitory either (data not shown) There was gradual decrease of stimulation with lower concentrations of LA and no stimu lation was seen with 0 5 μg/ml The dose re sponse curve of lymphocytes to Con A was similar except that the highest concentration 500 μg/ml was found to be inhibitory (Fig 1) The differences between the mitogens were probably related to the purity of reagents

Maximal stimulation ratios with each mito gen at optimal concentrations were similar Fig 1) Individual variation in the control subjects was such that 2 standard deviations of the mean almost equalled maximal stimula tion ratio

Table 2 *Lymphocyte responsiveness to leucoagglutinin in patients with pyelonephritis and LUTI at various stages of the infection*

Days after admission	LA stimulation ratio compared to controls	Number of patients with decreased response			
		Pyelonephritis (n=27)		LUTI (n=21)	
0	Below −2 0 S D	15/27	56%	1/21	5%
	Below −1 0 S D	14/27	52%	15/21	71%
14±	Below – 0 S D	0/27	0%	0/21	0%
	Below −1 0 S D	14/27	52%	12/21	52%
4 ±5	Below – 0 S D	2/2	7%	0/ 1	0%
	Below −1 0 S D	4/27	15%	7/ 1	33%

See text for explanation

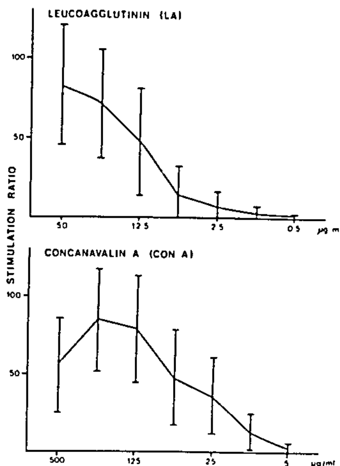


Fig. 1. Lymphocyte stimulation in whole blood cultures with different concentrations of leucoagglutinin and concanavalin A of 35 control children. The curves show mean stimulation ratios for each mitogen and the bars represent ±1 standard deviation of the mean.

urine specimens yielding bacterial growth of the same strain over 100000 per ml were required for diagnosis. Most patients were initially treated in the hospital but were discharged after the urine no longer yielded significant growth of bacteria. The therapeutic regimen varied according to the clinical picture, level diagnosis and bacteriological results.

In the pyelonephritis group the mean age was 5.0 years (range 2 months to 11 years 3 months) and in the LUTI group 5.4 years (range 2 months to 11 years 7 months). In 27 cases there were no previous histories of UTI; the remaining 21 patients (9 in the pyelonephritis group and 12 in the LUTI group) had had one or more previous episodes of UTI.

The first blood specimen for lymphocyte stimulation and other laboratory studies was collected prior to the start of antibiotic therapy. The second specimen was taken 14 days after the first; there was no more than 2 days variation in the collection of the specimens. A third specimen was taken 6 weeks after the first one.

The control group for the lymphocyte stimulation test consisted of 35 children with a mean age of 6.4 years (range 2 to 15 years). All were in good health, the majority coming to the hospital for elective surgery. The remainder were hospitalized for various investigations.

Lymphocyte stimulation test

The method was based on the whole blood culture technique with different concentrations of mitogens described by Park & Good (11) substituting gamma counting for liquid scintillation counting (4).

The cultures were established on U-bottom Cooke Microtiter® plates (M24 ARTL). Into each well 0.025 ml of freshly collected heparinized whole blood and 0.1 ml of Eagle's MEM (Orion Diagnostica) containing the mitogen were added. The cultures were set in triplicate for each concentration of the mitogens. The incubation time was 72 hours at 37°C in an atmosphere containing 5% CO_2. Six hours before harvest 0.125 µCi of ^{125}I iodo-2-deoxyuridine (Amersham) was added in 0.025 ml of MEM. The cells were collected using a multiple cell culture harvester (Skatron, Flow laboratories) on glassfibre discs which were dried and counted in a Wallac gamma counter.

Two mitogens were used: Leucoagglutinin (LA), a purified component of phytohemagglutinin (Pharmacia Fine Chemicals) and crude concanavalin A (Con A)

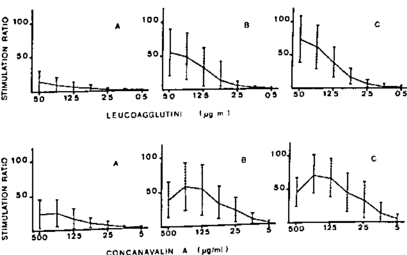


Fig. 2. Lymphocyte stimulation with leucoagglutinin (above) and concanavalin A (below) in 27 children with pyelonephritis. The curves show mean stimulation ratios and the bars ±1 S.D. of the mean. The dotted area represents ±1 S.D. of the control children. The lymphocyte stimulation was studied at the acute stage (A), at 2 weeks (B) and at 6 weeks (C) after infection.

experiments Miller et al noticed marked suppression of splenic T lymphocyte PHA responsiveness after experimental pyelonephritis In their model little change was seen in peripheral blood lymphocyte function (9) Williams et al found a clear correlation between renal abscess formation and decrease in splenic lymphocyte response to Con A In their study if artificial inoculation produced renal abscesses the lymphocyte stimulation indices were highly significantly reduced whereas in cases with kidney infection without abscesses there was only slight although significant suppression of lymphocyte responsiveness (12)

Our series of lymphocyte stimulation studies may represent a human counterpart for the immunosuppression observed in experimental pyelonephritis by Williams et al (12) One half of the patients with pyelonephritis showed almost total unresponsiveness of lymphocytes whereas in all of those with LUTI the immunosuppression was much less The criteria used by us in the level diagnosis of UTI were indirect measures of systemic response to infection and did not necessarily reflect actual pathology in the kidneys However if the observations from the animal model can be applied to human disease patients with seriously impaired CMI might also have abscess formation in the kidneys and the degree of acute stage immunosuppression in UTI could be an important indicator of the seriousness of the infection process at kidney level

After treatment of the infection the lymphocyte responses returned to normal in most patients There were however a few notable exceptions These were in most cases associated with recurrent UTI signs of chronic pyelonephritis or another infection elsewhere Consequently lymphocyte stimulation studies might be of significance in the monitoring of recovery and in detecting chronic infection It must be borne in mind however that suppression of lymphocyte mitogen responsiveness is not specific for UTI but may be associated with other viral or bacterial infection (7)

Since decreased lymphocyte responsiveness in the late convalescent stage in most cases was seen only together with an associated cause we believe that none of the patients had a primary defect of CMI It is difficult however to assess the role of impaired CMI in the recurrence of UTI A recurrent or chronic UTI may cause immunosuppression but CMI suppressed by other causes might precipitate recurrence

The present whole blood method for lymphocyte stimulation appears rapid and simple enough to include in the laboratory work up of patients with UTI Of the two mitogens used in the study LA is more suitable since it causes no agglutination of red blood cells Testing with different concentrations of a mitogen may not be necessary The suboptimal concentrations of LA and Con A were used in the study as it is known that a slight suppression of lymphocyte stimulation is more readily detected under such conditions (2 11) This was really not necessary in the present work as a marked immunosuppression was seen in most patients Furthermore the normal variation in the controls was of such a degree that a suspected slight decrease in lymphocyte responsiveness should not be given too much attention Although the present limits of −1 0 S D and −2 0 S D of the mean were arbitrary measures of decreased CMI they may represent right orders of magnitude for probable and significant immunosuppression respectively

The mechanism of impaired CMI in acute UTI remains unclear and was not elucidated to any greater degree in the present study However it did not appear to be due to serum factors since purified lymphocytes from a few patients also showed decreased responsiveness to LA when cultured in normal human serum or fetal calf serum (data not shown) This is in accordance with the results of Williams et al in the rat system (12)

In conclusion the whole blood culture method for lymphocyte stimulation with LA is feasible and may provide useful information

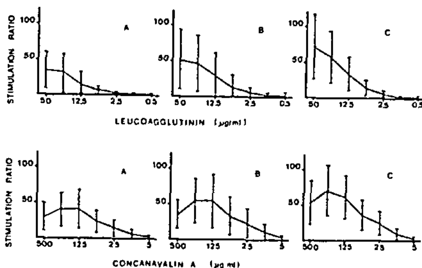


Fig 3 Lymphocyte stimulation with LA (above) and Con A (below) in 21 children with LUTI The curves show mean stimulation ratios and the bars ±1 S D of the mean The dotted area represents ±1 S D *of the control group of children* The lymphocyte stimulation was studied at the acute stage (A) at 2 weeks (B) and at 6 weeks (C) after infection

Lymphocyte stimulation in acute UTI At the acute stage of pyelonephritis the lymphocyte responses to LA were suppressed in all patients (<−1 0 S D of the control group) and the mean stimulation ratio of the group with the highest concentration of LA was below that of controls (15 7 vs 82 7 $p<0\ 001$) The mean s r to Con A was similarly suppressed (Fig 2)

The shape of the dose response curve was also changed when maximal responsiveness was decreased This was best seen in the Con A stimulated cultures at the acute stage of pyelonephritis as the best responses were obtained using Con A at a concentration of 500 µg/ml which in normal conditions was inhibitory (Fig 2)

At 2 weeks the lymphocyte responses had become normal in approximately one half of the patients (Table 2) but the mean stimulation ratio of the group was still below control level By 6 weeks there was no difference in the mean s r compared to controls (Fig 2) although 6 individuals showed impaired lymphocyte responsiveness at this stage

In most patients with LUTI lymphocyte responses were suppressed in the acute stage (Table 2) but the mean stimulation ratio of the group was only approximately minus 1 0 standard deviations below the controls There was some improvement of lymphocyte responsiveness by 2 weeks and by 6 weeks the mean stimulation ratio did not differ significantly from controls (Fig 3) but there were 7 individuals still with somewhat decreased lymphocyte responsiveness

Lymphocyte stimulation in the level diagnosis of UTI Table 1 shows how the standard criteria for the diagnosis of pyelonephritis were met In most cases there was no ambiguity in differentiating pyelonephritis from LUTI Although there was no clear cut differences between the groups in the lymphocyte responsiveness it appeared that lymphocyte stimulation ratio below minus 2 0 standard deviations from the controls approaching anergy was suggestive of pyelonephritis and correlated with the other criteria for systemic response to infection

By 6 weeks at which time the initial infection had been treated in all cases there were still 13 patients in whom the lymphocyte stimulation ratio was below −1 0 S D of the control group Among these patients 1 had a recurrent pyelonephritis and 2 had a recurrent LUTI Two more patients had another febrile infection Four patients had X ray findings suggestive of chronic pyelonephritis and 1 had crossed ectopia of the right kidney In the remaining 3 there was no apparent associated condition

DISCUSSION

The present results indicating suppression of CMI at the acute stage of urinary tract infections are supported by observations in animal

experiments Miller et al noticed marked suppression of splenic T lymphocyte PHA responsiveness after experimental pyelonephritis In their model little change was seen in peripheral blood lymphocyte function (9) Williams et al found a clear correlation between renal abscess formation and decrease in splenic lymphocyte response to Con A In their study if artificial inoculation produced renal abscesses the lymphocyte stimulation indices were highly significantly reduced whereas in cases with kidney infection without abscesses there was only slight although significant suppression of lymphocyte responsiveness (12)

Our series of lymphocyte stimulation studies may represent a human counterpart for the immunosuppression observed in experimental pyelonephritis by Williams et al (12) One half of the patients with pyelonephritis showed almost total unresponsiveness of lymphocytes whereas in all of those with LUTI the immunosuppression was much less The criteria used by us in the level diagnosis of UTI were indirect measures of systemic response to infection and did not necessarily reflect actual pathology in the kidneys However if the observations from the animal model can be applied to human disease patients with seriously impaired CMI might also have abscess formation in the kidneys and the degree of acute stage immunosuppression in UTI could be an important indicator of the seriousness of the infection process at kidney level

After treatment of the infection the lymphocyte responses returned to normal in most patients There were however a few notable exceptions These were in most cases associated with recurrent UTI signs of chronic pyelonephritis or another infection elsewhere Consequently lymphocyte stimulation studies might be of significance in the monitoring of recovery and in detecting chronic infection It must be borne in mind however that suppression of lymphocyte mitogen responsiveness is not specific for UTI but may be associated with other viral or bacterial infection (7)

Since decreased lymphocyte responsiveness in the late convalescent stage in most cases was seen only together with an associated cause we believe that none of the patients had a primary defect of CMI It is difficult however to assess the role of impaired CMI in the recurrence of UTI A recurrent or chronic UTI may cause immunosuppression but CMI suppressed by other causes might precipitate recurrence

The present whole blood method for lymphocyte stimulation appears rapid and simple enough to include in the laboratory work up of patients with UTI Of the two mitogens used in the study LA is more suitable since it causes no agglutination of red blood cells Testing with different concentrations of a mitogen may not be necessary The suboptimal concentrations of LA and Con A were used in the study as it is known that a slight suppression of lymphocyte stimulation is more readily detected under such conditions (2 11) This was really not necessary in the present work as a marked immunosuppression was seen in most patients Furthermore the normal variation in the controls was of such a degree that a suspected slight decrease in lymphocyte responsiveness should not be given too much attention Although the present limits of $-1\ 0$ S D and $-2\ 0$ S D of the mean were arbitrary measures of decreased CMI they may represent right orders of magnitude for probable and significant immunosuppression respectively

The mechanism of impaired CMI in acute UTI remains unclear and was not elucidated to any greater degree in the present study However it did not appear to be due to serum factors since purified lymphocytes from a few patients also showed decreased responsiveness to LA when cultured in normal human serum or fetal calf serum (data not shown) This is in accordance with the results of Williams et al in the rat system (12)

In conclusion the whole blood culture method for lymphocyte stimulation with LA is feasible and may provide useful information

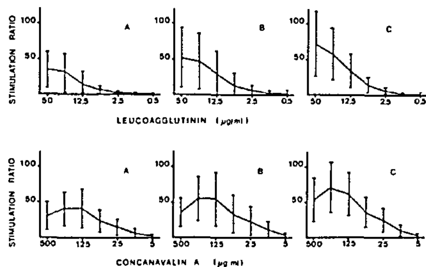


Fig 3 Lymphocyte stimulation with LA (above) and Con A (below) in 21 children with LUTI The curves show mean stimulation ratios and the bars ±1 S D of the mean The dotted area represents ±1 S D of the control group of children The lymphocyte stimulation was studied at the acute stage (A) at 2 weeks (B) and at 6 weeks (C) after infection

Lymphocyte stimulation in acute UTI At the acute stage of pyelonephritis the lymphocyte responses to LA were suppressed in all patients (<−1 0 S D of the control group) and the mean stimulation ratio of the group with the highest concentration of LA was below that of controls (15 7 vs 82 7 $p<0\ 001$) The mean s r to Con A was similarly suppressed (Fig 2)

The shape of the dose response curve was also changed when maximal responsiveness was decreased This was best seen in the Con A stimulated cultures at the acute stage of pyelonephritis as the best responses were obtained using Con A at a concentration of 500 µg/ml which in normal conditions was inhibitory (Fig 2)

At 2 weeks the lymphocyte responses had become normal in approximately one half of the patients (Table 2) but the mean stimulation ratio of the group was still below control level By 6 weeks there was no difference in the mean s r compared to controls (Fig 2) although 6 individuals showed impaired lymphocyte responsiveness at this stage

In most patients with LUTI lymphocyte responses were suppressed in the acute stage (Table 2) but the mean stimulation ratio of the group was only approximately minus 1 0 standard deviations below the controls There was some improvement of lymphocyte responsiveness by 2 weeks and by 6 weeks the mean stimulation ratio did not differ significantly from controls (Fig 3) but there were 7 individuals still with somewhat decreased lymphocyte responsiveness

Lymphocyte stimulation in the level diagnosis of UTI Table 1 shows how the standard criteria for the diagnosis of pyelonephritis were met In most cases there was no ambiguity in differentiating pyelonephritis from LUTI Although there was no clear cut differences between the groups in the lymphocyte responsiveness it appeared that lymphocyte stimulation ratio below minus 2 0 standard deviations from the controls approaching anergy was suggestive of pyelonephritis and correlated with the other criteria for systemic response to infection

By 6 weeks at which time the initial infection had been treated in all cases there were still 13 patients in whom the lymphocyte stimulation ratio was below −1 0 S D of the control group Among these patients 1 had a recurrent pyelonephritis and 2 had a recurrent LUTI Two more patients had another febrile infection Four patients had X ray findings suggestive of chronic pyelonephritis and 1 had crossed ectopia of the right kidney In the remaining 3 there was no apparent associated condition

DISCUSSION

The present results indicating suppression of CMI at the acute stage of urinary tract infections are supported by observations in animal

Acta Pædiatr Scand 68 507–512 1979

SEROLOGICAL DIFFERENTIATION OF CONGENITAL AND ACQUIRED CYTOMEGALOVIRUS INFECTIONS DETECTED IN INFANCY

K AHLFORS S A IVARSSON and T JOHNSSON

From the Department of Clinical Virology and the Department of Paediatrics University of Lund Malmo General Hospital Malmo Sweden

ABSTRACT Ahlfors K Ivarsson S A and Johnsson T (Department of Clinical Virology and Department of Paediatrics University of Lund Malmo General Hospital Malmo Sweden) Serological differentiation of congenital and acquired cytomegalovirus infections detected in infancy Acta Paediatr Scand 68 507 1979 —A serological investigation on infants with incidentally detected cytomegalovirus (CMV) excretion was made in an attempt to differentiate between congenital and acquired infections Generally each of the patients 0–12 months old at the detection of CMV-excretion was studied by indirect immunofluorescence (IIF) test for IgM antibodies in cord serum and by complement fixation (CF) test performed on cord serum and a number of sera drawn after the detection of CMV Two out of 4 patients with virologically confirmed congenital CMV infection as shown by positive virus isolation within 1 week of age had a positive CMV IgM test in cord serum One of these two children also had a persistently high CMV CF titer from birth until 2 months of age indicating congenital infection In the remaining 46 cases all with the CMV-excretion detected after 3 weeks of age in half of the cases after 5 months no positive IgM reaction was recorded in cord serum No persistently high CF titer could be demonstrated among 13 out of the 46 patients from whom sera were drawn at birth and at 1–4 months Six out of these 13 patients had a CF titer rise after the period of 1–4 months indicating acquired infection However also one of the congenitally infected children had a similar titer increase Many patients lacked characteristic serological patterns some of them in spite of access to sera drawn at birth as well as at 1–4 months of age and later on It could be concluded that the possibility of making a serological distinction of congenital and acquired infant CMV infection found by chance during infancy is limited

KEY WORDS Cytomegalovirus infection serology congenital neonatal acquired

Because of frequent often unexpected finding of cytomegalovirus (CMV) infection among children treated at Malmo General Hospital there were two recurrent questions first is the infection congenital with possible sequelae as result second does the finding have any causal relationship to the patient s clinical symptoms In order to analyse these questions 661 consecutive children 0–12 months old were studied by virus isolation at admittance to hospital (1) It was shown that CMV uria occurred among 1% of the infants less than one month old and among 23% of the older children As a rule there was no clinical suspicion of CMV infection From the virological studies it was concluded that CMV uria before 2–3 weeks of age could be considered proof of congenital infection while thereafter it usually depended on natal/postnatal virus transmission However in order to further analyse the patients with later detected infection serological studies were performed The present study illustrates the difficulties in distinguishing between the congenitally and natally/postnatally infected CMV excreters found by chance in practical paediatric work

MATERIAL AND METHODS

Four congenitally infected children Among the 661 patients studied four were shown to have CMV uria within the above mentioned critical period of 2–3 weeks of age in fact within one week Only one of them had typical CMV symptoms at birth and neurological sequelae (1) Cord serum was drawn from each of these four patients and a total of 19 further sera were taken up to

of the patient s condition both at the acute and convalescent stages of UTI Suppression of CMI appears to be of value in the level diag nosis of UTI The role of cellular immuno suppression in the pathogenesis of recurrent infections remains to be established

ACKNOWLEDGEMENTS

The technical assistance of Miss Outi Karkkainen and Mrs Taimi Tammipuu are gratefully acknowledged

REFERENCES

1 Anttila R Gröhn P & Krohn K Transfer factor and cellular immune response in urinary tract infec tion in children *Acta Paediatr Scand* 66 219 1977
2 Buimovici Klein E Vesikari T Santangelo C F & Cooper L Z Study of the lymphocyte in vitro response to rubella antigen and phytohemagglutinin by a whole blood method *Arch Virol* 52 323 1976
3 Dubroff L M Duckett J W & Corriere J N Jr Phytohemagglutinin lymphocyte stimulation in chil dren with recurrent urinary tract infections *Urology* 5 744 1975
4 Eskola J Soppi E Viljanen M & Ruuskanen O A new micromethod for lymphocyte stimulation using whole blood *Immunol Commun* 4 297 1975
5 Hanson L Å Ahlstedt S Fasth A Jodal U Kaijser B Larsson P Lindberg U Olling S Sohl Åkerlund A & Svanborg Edén C *Antigens of* Escherichia coli human immune response and the pathogenesis of urinary tract infections *J Infect Dis 136* Suppl 144 1977
6 Jodal U Lindberg U & Lincoln K Level diag nosis of symptomatic urinary tract infections in childhood *Acta Paediatr Scand* 64 201 1975
7 Kantor F S Infection anergy and cell mediated immunity *N Engl J Med* 292 629 1975
8 Kouvalainen K Pelkonen P & Parkkulainen K V Immunoglobulins in chronic urinary tract infec tions in children *Ann Clin Res* 1 187 1969
9 Miller T E Simpson G & Ormrod D J Quanti tation of immunoglobulin bearing lymphocytes and the lymphocyte response to PHA in experimental pyelonephritis *Clin Exp Immunol* 21 474 1975
10 Nilsson L Å Comparative testing of precipitation methods for quantitation of C reactive protein in blood serum *Acta Pathol Microbiol Scand* 73 129 1968
11 Park B H & Good R A A new micromethod for evaluating lymphocyte responses to phytohemag glutinin Quantitative analysis of the function of thymus dependent cells *Proc Acad Sci USA* 69 371 1972
12 Williams T W Friedlander A M Lyons J M & Brande A J Cellular immunity in pyelonephritis inhibition of the spleen cell response to the mitogen Concanavalin A *Cell Immunol* 14 33 1974
13 Winberg J Determination of renal concentration capacity in infants and children without renal disease *Acta Paediatr Scand* 48 318 1959
14 Winberg J Andersen H J Bergstrom T Jacobs son B Larson H & Lincoln K Epidemiology of symptomatic urinary tract infection in childhood *Acta Paediatr Scand* Suppl 252 1 1974

Submitted Oct 30 1978
Accepted Jan 26 1979

(M J) Department of Paediatrics
Tampere Central Hospital
33520 Tampere 52
Finland

A

Acta Pædiatr Scand 68 507–512 1979

SEROLOGICAL DIFFERENTIATION OF CONGENITAL AND ACQUIRED CYTOMEGALOVIRUS INFECTIONS DETECTED IN INFANCY

K AHLFORS S A IVARSSON and T JOHNSSON

From the Department of Clinical Virology and the Department of Paediatrics University of Lund Malmo General Hospital Malmo Sweden

ABSTRACT Ahlfors K Ivarsson S A and Johnsson T (Department of Clinical Virology and Department of Paediatrics University of Lund Malmo General Hospital Malmo Sweden) Serological differentiation of congenital and acquired cytomegalovirus infections detected in infancy Acta Paediatr Scand 68 507 1979.—A serological investigation on infants with incidentally detected cytomegalovirus (CMV) excretion was made in an attempt to differentiate between congenital and acquired infections Generally each of the patients 0–12 months old at the detection of CMV-excretion was studied by indirect immunofluorescence (IIF) test for IgM antibodies in cord serum and by complement fixation (CF) test performed on cord serum and a number of sera drawn after the detection of CMV Two out of 4 patients with virologically confirmed congenital CMV infection as shown by positive virus isolation within 1 week of age had a positive CMV IgM test in cord serum One of these two children also had a persistently high CMV CF titer from birth until 2 months of age indicating congenital infection In the remaining 46 cases all with the CMV-excretion detected after 3 weeks of age in half of the cases after 5 months no positive IgM reaction was recorded in cord serum No persistently high CF titer could be demonstrated among 13 out of the 46 patients from whom sera were drawn at birth and at 1–4 months Six out of these 13 patients had a CF titer rise after the period of 1–4 months indicating acquired infection However also one of the congenitally infected children had a similar titer increase Many patients lacked characteristic serological patterns some of them in spite of access to sera drawn at birth as well as at 1–4 months of age and later on It could be concluded that the possibility of making a serological distinction of congenital and acquired infant CMV infection found by chance during infancy is limited

KEY WORDS Cytomegalovirus infection serology congenital neonatal acquired

Because of frequent often unexpected finding of cytomegalovirus (CMV) infection among children treated at Malmo General Hospital there were two recurrent questions first is the infection congenital with possible sequelae as result second does the finding have any causal relationship to the patient s clinical symptoms In order to analyse these questions 661 consecutive children 0–12 months old were studied by virus isolation at admittance to hospital (1) It was shown that CMV uria occurred among 1% of the infants less than one month old and among 23% of the older children As a rule there was no clinical suspicion of CMV infection From the virological studies it was concluded that CMV uria before 2–3 weeks of age could be considered proof of congenital infection while thereafter it usually depended on natal/postnatal virus transmission However in order to further analyse the patients with later detected infection serological studies were performed The present study illustrates the difficulties in distinguishing between the congenitally and natally/postnatally infected CMV excreters found by chance in practical paediatric work

MATERIAL AND METHODS

Four congenitally infected children Among the 661 patients studied four were shown to have CMV uria within the above mentioned critical period of 2–3 weeks of age in fact within one week Only one of them had typical CMV symptoms at birth and neurological sequelae (1) Cord serum was drawn from each of these four patients and a total of 19 further sera were taken up to

of the patient s condition both at the acute and convalescent stages of UTI Suppression of CMI appears to be of value in the level diag nosis of UTI The role of cellular immuno suppression in the pathogenesis of recurrent infections remains to be established

ACKNOWLEDGEMENTS

The technical assistance of Miss Outi Karkkainen and Mrs Taimi Tammipuu are gratefully acknowledged

REFERENCES

1 Anttila R Grohn P & Krohn K Transfer factor and cellular immune response in urinary tract infec tion in children *Acta Paediatr Scand* 66 219 1977
2 Buimovici Klein E Vesikari T Santangelo C F & Cooper L Z Study of the lymphocyte in vitro response to rubella antigen and phytohemagglutinin by a whole blood method *Arch Virol* 52 323 1976
3 Dubroff L M Duckett J W & Corriere J N Jr Phytohemagglutinin lymphocyte stimulation in chil dren with recurrent urinary tract infections *Urology* 5 744 1975
4 Eskola J Soppi E Viljanen M & Ruuskanen O A new micromethod for lymphocyte stimulation using whole blood *Immunol Commun* 4 297 1975
5 Hanson L Å Ahlstedt S Fasth A Jodal U Kaijser B Larsson P Lindberg U Olling S Sohl Åkerlund A & Svanborg Edén C Antigens of Escherichia coli human immune response and the pathogenesis of urinary tract infections *J Infect Dis* 136 Suppl 144 1977
6 Jodal U Lindberg U & Lincoln K Level diag nosis of symptomatic urinary tract infections in childhood *Acta Paediatr Scand* 64 201 1975
7 Kantor F S Infection anergy and cell mediated immunity *N Engl J Med* 292 679 1975
8 Kouvalainen K Pelkonen P & Parkkulainen K V Immunoglobulins in chronic urinary tract infec tions in children *Ann Clin Res* 1 182 1969
9 Miller T E Simpson G & Ormrod D J Quanti tation of immunoglobulin bearing lymphocytes and the lymphocyte response to PHA in experimental pyelonephritis *Clin Exp Immunol* 21 474 1975
10 Nilsson L Å Comparative testing of precipitation methods for quantitation of C reactive protein in blood serum *Acta Pathol Microbiol Scand* 73 179 1968
11 Park B H & Good R A A new micromethod for evaluating lymphocyte responses to phytohemag glutinin Quantitative analysis of the function of thymus dependent cells *Proc Acad Sci USA* 69 371 1972
12 Williams T W Friedlander A M Lyons J M & Brande A J Cellular immunity in pyelonephritis inhibition of the spleen cell response to the mitogen Concanavalin A *Cell Immunol* 14 33 1974
13 Winberg J Determination of renal concentration capacity in infants and children without renal disease *Acta Paediatr Scand* 48 318 1959
14 Winberg J Andersen H J Bergstrom T Jacobs son B Larson H & Lincoln K Epidemiology of symptomatic urinary tract infection in childhood *Acta Paediatr Scand* Suppl 252 1 1974

Submitted Oct 30 1978
Accepted Jan 26 1979

(M J) Department of Paediatrics
Tampere Central Hospital
33520 Tampere 52
Finland

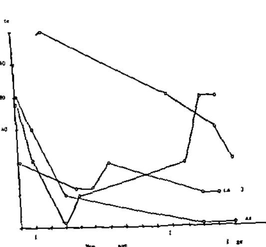
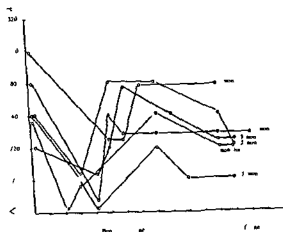


Fig 1 CF antibody development of congenitally infected patients

mentioned fourth case the CF titer at 1–4 months of age was high (1/320) which was comparable to the maternal titer 1/160 at delivery After the period of 1–4 months two patients had falling levels which in one case ended in a titer ≤1/5 one patient had a persistently low titer (1/20–1/10) and another one a marked CF titer rise

CF antibodies among infants with CMVuria detected after 3 weeks of age At birth 43/45 (96%) patients tested had a positive CMV CF titer varying between 1/10–1/320 with the geometric mean (GM) titer 1/40 The CF titer development of the two patients with negative test (CF titer ≤1/5) in cord serum born by CF negative mothers was not followed up Virus isolations from these two patients were positive in the fourth week and at ten months of age On comparison of sera drawn at birth and at 1–4 months 11/13 infants with CMV excretion detected at 1–4 months of age had falling titers while the remaining two showed persistently low titers (≤1/40) After the age of four months six patients with CMVuria at 1–4 months showed significant titer increases (Fig 2A) Five other patients with primary CMV isolation at 1–10 months and sera drawn at 1–4 months and later on did not show any postnatal titer rise (Fig 2B) For the 19 children



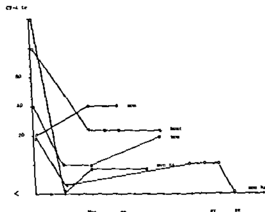


Fig 2 Individual CF titer development of patients with CMV infection detected after 3 weeks of age The age of the patients at the detection of viruria is noticed (A) Patients with CF titer rise (B) Patients without CF titer rise

admitted to hospital after five months of age sera drawn at 1–4 months and in the acute phase of the disease usually were missing In spite of continued virus excretion five patients showed a significant CF titer decrease after 7–8 months of age one of them to titer ≤1/5 At 2–3 years 4/16 (25%) of the children tested did not have measurable amounts of CF antibodies

CF antibodies among mothers of congenitally infected children The distribution of maternal CMV CF titers at delivery in this and the two following paragraphs is shown in Fig 3 All mothers of the congenitally infected infants had positive CF titers at the

3 years of age The cord sera were studied by indirect immunofluorescence (IIF) test for IgM antibodies and all sera but one cord sample by complement fixation (CF) test

Forty six children with CMV uria detected after 3 weeks of age Out of the 661 infants tested 57 were more than 3 weeks old at the detection of CMV uria almost half of them in fact more than 5 months Only forty six of them were studied by serological methods Different symptoms of infection predominated *in the entire patient* material as well as among the CMV excreters (1) Forty five cord sera were taken and 103 sera from one month up to three years of age 1–8 per individual Few post natal sera were taken until the virus isolation result was evident The cord sera were—with few exceptions—tested by IgM IIF and all sera by CF

Randomized cord sera for control of the IgM IIF technique One hundred cord sera from randomized babies were tested by the IgM IIF technique for specific and unspecific reactions The clinical status of these babies was not analysed

Serum panel for control of the CF technique A panel of 14 sera kindly supplied by professor U H Krech Schweiz was tested by CF at 14 laboratories the present one included

Mothers of the four congenitally infected children In all cases maternal sera were drawn at delivery The samples were tested by CF

Mothers of the 46 children with CMV uria detected after 3 weeks of age Sera were drawn from 42/46 mothers at delivery They were studied by CF

Control group of 348 randomized mothers This group was studied in order to obtain information on the CMV CF status at delivery in general

Sera Sera had been stored for a varying period of time up to five years at −20°C

Indirect immunofluorescence test for IgM antibodies in cord sera Slide cultures of human embryonal lung fibroblasts grown in Eagle s MEM with 10% fetal calf serum and antibiotics and maintained in Eagle s MEM with 3% fetal calf serum and antibiotics were inoculated with CMV AD 169 incubated in CO_2 atmosphere at 35 C and 3–5 days after the appearance of cytopathogenic effect washed in phosphate buffer and fixed in acetone at 4 C for 10 min The slides were stored at −20 C for at least one week before being tested They were stable for months Before use they were washed in distilled water Cord sera were tested concomitantly on two different batches of slides at dilutions 1/4 and 1/16 and incubated at 35 C for 3 hours Two positive serum controls one at low dilution giving a strong fluorescence and one at high dilution near the positive end point were included on each slide together with a negative serum control with a high CMV IgG titer After a rapid rinsing in phosphate buffer pH 7 2 followed by a thorough washing with magnetic stirrer in the same type of buffer and a short rinsing in distilled water the slides were incubated for 1 hour with a specific FITC conjugated rabbit anti human (anti IgM heavy chains) serum from Dakopatts A/S Copenhagen Denmark diluted 1/15 After washing as before the slides were *mounted in* glycerine buffer pH 7 8 A Leitz fluorescence microscope with transmitted light was used for reading Only the fluorescence of intranuclear inclusions graded from 1+ to 3+ was recorded (8 13) Sera positive at the screening were retested at two-fold serial dilutions

Test for rheuma factors IgM IIF positive sera were tested for rheuma factors (RF) with the Latex RF Reagenz Behringwerke Germany at dilution 1/2 1/20 (5 12 14)

Complement fixation test CMV CF test which mainly reflects the IgG antibody status (3 4 6 7) was performed by a microtiter technique using 4–6 units of sonicated and heated (56 C 60 min) CMV AD-169 crude cell extract antigen and 2 units of complement Complement pretreatment was performed on all sera in order to prevent anticomplementary activity which was a common phenomenon (17) A fourfold CF titer increase or decrease was considered significant titers ≤1/5 negative and ≥1/10 positive Maternal and infant sera were tested in the same CF run

RESULTS

IgM antibodies in cord sera from congenitally infected children Two out of the four congenitally infected children had a positive IgM IIF reaction in cord serum both with titers 1/32 Both were RF negative when tested at serum dilution 1/2 and 1/20 respectively One of the IgM positive children had a typical CMV disease the other one was asymptomatic

IgM antibodies in cord sera from infants with CMV uria detected after 3 weeks of age Thirty nine out of the 46 children in this group were tested for CMV IgM antibodies in cord serum In all cases the IgM IIF titer was negative that is <1/4

Randomized cord sera for control of the IgM IIF technique All 100 randomized cord sera had CMV IgM titers <1/4 The cell nuclei appeared as black holes in a faint fluorescing cytoplasma

CF antibody development among congenitally infected children Three patients had a positive CMV CF titer in cord serum varying between *1/20–1/80* in the fourth case *no* CF test was performed (Fig 1) On comparison of cord sera and samples drawn at 1–4 months of age two patients showed significantly falling titers and the third a persistently low titer (1/20–1/10) In the above

noted by others (2 9 16) It is probable that the mother is the source not only of the congenital but also of most acquired infant infections

From the paper by Stagno et al it is evident that studies of the infant CF antibody development should include a serum sample drawn at 1–4 months of age (15) The average CF antibody titer of congenitally infected patients is then comparatively high in contrast to that of neonatally infected babies However as shown in the present study only one out of the four congenitally infected infants followed the expected pattern (Case 1 Fig 1) Born to a mother with CF titer 1/160 and with its own CF titer 1/320 at 2 months of age this child probably had had a persistently high titer since birth It might be of interest to add that this child was the only one who was symptomatic at birth and developed sequelae The absence of persistently high titers among patients with supposedly acquired infection does not exclude the possibility that some of them were congenitally infected as shown above

Six patients with CMV infection detected after 3 weeks of age had a postnatal CF titer rise a pattern which according to the previous observations by Stagno et al should be typical for acquired infection (15) However since also one of the congenitally infected patients in the present material—and two patients in the material of Melish & Hanshaw (11)—had such titer increases there obviously are exceptions to the rule Five other patients with supposed acquired infection and adequately drawn sera did not have postnatal titer increase The connection between titer rise and current disease in the above mentioned cases is an open question

Follow up until 3 years of age showed that 5/20 (20%) of the patients tested—one with congenital and four with probably acquired infections—lost their CF antibodies Thus a negative CF test performed on an older child or an adult person does not exclude the possibility of previous CMV infection and CF seroconversion later in life does not necessarily mean primary CMV infection Possibly more sensitive techniques like the enzyme linked immunosorbent assay might have shown residual antibodies

In summary only two out of the four cases with confirmed congenital infection would have been diagnosed by the demonstration of specific IgM antibodies in cord serum In one of these cases the diagnosis would have been indicated by a persistently high CF titer at 1–4 months of age In 6/46 cases with supposed acquired CMV infection the proposed diagnosis was supported by a postnatal CF titer rise In the remaining 40 cases the type of infection remained doubtful in some cases because of the absence of typical serological patterns specific IgM antibodies at birth persistently high CF antibody titers from birth until 1–4 months or CF titer increases In other cases sera drawn at the proper times were missing The present study has shown that the possibility of a serological diagnosis of congenital CMV infection is rather limited especially if cord serum is not available

ACKNOWLEDGEMENT

The authors wish to thank Mrs Cecilia Andersson Mrs Elisabeth Nilehn and Mrs Eva Nordstrom for their skillful technical assistance

REFERENCES

1 Ahlfors K Ivarsson S A Johnsson T & Svensson I Congenital and acquired cytomegalovirus infections Virological and clinical studies on a Swedish infant population *Acta Paediat Scand 67* 321 1978
2 Andersen H K Gravesen J J & Iversen T Cytomegalovirus infection among infants admitted to a paediatri department *Acta Paediatr Scand 61* 445 1972
3 Bernstein M T & Stewart J A Indirect hemagglutination test for detection of antibodies to cytomegalovirus *Appl Microbiol 21* 84 1971
4 Cremer N E Schmidt N J Jensen F Hoffman M Oshiro L S & Lenette E H Complement fixing antibody in human sera reactive with viral and soluble antigens of cytomegalovirus *J Clin Microbiol 1* 262 1975

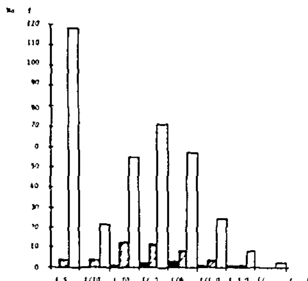


Fig. 3. Distribution of CF titers at delivery among mothers of congenitally infected children ■, mothers of children with probable natally/neonatally acquired infection ▨ and randomized control mothers □.

delivery with the GM titer 1/80. In the three cases studied the maternal and infant titers were equal at birth (a difference of ≤ one titer step).

CF antibodies among mothers of children with CMV uria detected after 3 weeks of age. Thirty nine out of 42 (93%) mothers tested had positive CMV CF reactions at the delivery with the GM titer 1/40. In 4/37 cases tested the infant titer level at birth exceeded the maternal titer with two steps, while in the other 33 cases the titers were equal.

CF antibodies in a control group of randomized mothers. Among 348 mothers tested 237 (68%) had positive CMV CF reactions at delivery with the GM titer 1/40.

Serum panel for control of the CF technique. According to a compilation by professor Krech there was almost complete agreement (95%) between the different laboratories regarding the outcome of the CMV CF tests—positive or negative. In no case did the outcome of the present laboratory diverge from the common results. The highest titers were obtained at this and five other laboratories.

DISCUSSION

In the present serological investigation the starting point was a positive CMV isolation from a patient at any time between birth and one year of age. Access to frozen cord sera was of primary importance. More sera drawn at 1–4 months and in the acute stage of the disease would have been desirable. Previous serological studies on CMV infected infants have mainly been prospective, starting directly after birth (11, 15). Thus Melish & Hanshaw (11) studied the occurrence of CMV IgM antibodies among a series of newborn infants and the CF titer development of the congenitally infected babies detected. Stagno et al. (15) followed from birth the antibody development of infants congenitally or neonatally infected by CMV.

In the present study only two out of the four congenitally infected children—the diagnoses confirmed by positive virus isolation within one week of age—had positive IgM IIF tests at birth as a serological sign of the intrauterine infection. One of these two patients was symptomatic at birth, the other asymptomatic. It is well known that congenitally infected children often do not have CMV IgM antibodies at birth (10, 11). According to Luthardt (10) there is no correlation between the occurrence of IgM antibodies and the gravity of the disease. It can be concluded that some children with CMV uria detected after 3 weeks of age can have been congenitally infected in spite of the absence of positive IgM reactions at birth.

Determination of the infant—or the maternal—CF antibody status at birth was shown to be of little differential diagnostic value since all congenitally infected infants tested as well as 43/45 (96%) of those with probable acquired infection had a positive reaction. When the mothers of the children with supposed acquired infection were compared to mothers in the general population 39/42 (93%) were CMV CF positive in the former group and 237/348 (68%) in the latter group, a difference which is statistically significant ($p < 0.001$). The above mentioned finding that children infected by CMV as a rule are born to seropositive mothers had already been

noted by others (2 9 16) It is probable that the mother is the source not only of the congenital but also of most acquired infant infections

From the paper by Stagno et al it is evident that studies of the infant CF antibody development should include a serum sample drawn at 1–4 months of age (15) The average CF antibody titer of congenitally infected patients is then comparatively high in contrast to that of neonatally infected babies However as shown in the present study only one out of the four congenitally infected infants followed the expected pattern (Case 1 Fig 1) Born to a mother with CF titer 1/160 and with its own CF titer 1/320 at 2 months of age this child probably had had a persistently high titer since birth It might be of interest to add that this child was the only one who was symptomatic at birth and developed sequelae The absence of persistently high titers among patients with supposedly acquired infection does not exclude the possibility that some of them were congenitally infected as shown above

Six patients with CMV infection detected after 3 weeks of age had a postnatal CF titer rise a pattern which according to the previous observations by Stagno et al should be typical for acquired infection (15) However since also one of the congenitally infected patients in the present material—and two patients in the material of Melish & Hanshaw (11)—had such titer increases there obviously are exceptions to the rule Five other patients with supposed acquired infection and adequately drawn sera did not have postnatal titer increase The connection between titer rise and current disease in the above mentioned cases is an open question

Follow up until 3 years of age showed that 5/20 (20%) of the patients tested—one with congenital and four with probably acquired infections—lost their CF antibodies Thus a negative CF test performed on an older child or an adult person does not exclude the possibility of previous CMV infection and CF seroconversion later in life does not necessarily mean primary CMV infection Possibly more sensitive techniques like the enzyme linked immunosorbent assay might have shown residual antibodies

In summary only two out of the four cases with confirmed congenital infection would have been diagnosed by the demonstration of specific IgM antibodies in cord serum In one of these cases the diagnosis would have been indicated by a persistently high CF titer at 1–4 months of age In 6/46 cases with supposed acquired CMV infection the proposed diagnosis was supported by a postnatal CF titer rise In the remaining 40 cases the type of infection remained doubtful in some cases because of the absence of typical serological patterns specific IgM antibodies at birth persistently high CF antibody titers from birth until 1–4 months or CF titer increases In other cases sera drawn at the proper times were missing The present study has shown that the possibility of a serological diagnosis of congenital CMV infection is rather limited especially if cord serum is not available

ACKNOWLEDGEMENT

The authors wish to thank Mrs Cecilia Andersson Mrs Elisabeth Nilehn and Mrs Eva Nordstrom for their skillful technical assistance

REFERENCES

1 Ahlfors K Ivarsson S A Johnsson T & Svensson I Congenital and acquired cytomegalovirus infections Virological and clinical studies on a Swedish infant population *Acta Paediatr Scand 67* 321 1978
2 Andersen H K Gravesen J J & Iversen T Cytomegalovirus infection among infants admitted to a paediatric department *Acta Paediatr Scand 61* 445 1972
3 Bernstein M T & Stewart J A Indirect hemagglutination test for detection of antibodies to cytomegalovirus *Appl Microbiol 21* 84 1971
4 Cremer N E Schmidt N J Jensen F Hoffman M Oshiro L S & Lenette E H Complement fixing antibody in human sera reactive with viral and soluble antigens of cytomegalovirus *J Clin Microbiol 1* 262 1975

5 Fraser K B Shirodaria P V & Stanford C F Fluorescent staining and human IgM *Br Med J III* 707 1971
6 Hanshaw J B Congenital cytomegalovirus infection laboratory methods of detection *J Pediatr 75* 1179 1969
7 Lang D J & Noren B Cytomegaloviremia following congenital infection *J Pediatr 73* 812 1968
8 Langenhuysen M M A C The T H Nieweg H O & Kapsenberg J G Demonstration of IgM cytomegalovirus antibodies as an aid to early diagnosis in adults *Clin Exp Immunol* 6 387 1970
9 Leinikki P Granstrom M L Santavuori P & Pettay O Epidemiology of cytomegalovirus infections during pregnancy and infancy A prospective study *Scand J Infect Dis 10* 165 1978
10 Luthardt T Cytomegalie *In* O Vivell & W Burmeister (eds) *Klinische Padiatrie* Beihefte Ferdinand Enke Verlag Stuttgart 1976 p 14
11 Melish M E & Hanshaw J B Congenital cytomegalovirus infection Developmental progress of infants detected by routine screening *Am J Dis Child 126* 190 1973
12 Reimer C B Black C M Philips D J Logan L C Hunter E F Pender B J & McGrew B F The specificity of fetal IgM antibody or anti antibody? *Ann NY Acad Sci 254* 77 1975
13 Schmitz H & Haas R Determination of different cytomegalovirus immunoglobulins (IgG IgA IgM) by immunofluorescence *Arch Gesamte Virusforsch 37* 131 1972
14 Shirodaria P V Fraser K B & Stanford F Secondary fluorescent staining of virus antigens by rheumatoid factor and fluorescein conjugated anti IgM *Ann Rheum Dis 32* 53 1973
15 Stagno S Reynolds D W Tsiantos A Fucillo D A Long W & Alford C A Comparative serial virologic and serologic studies of symptomatic and subclinical congenitally and natally acquired cytomegalovirus infections *J Infect Dis 132* 568 1975
16 Stoll H U Zytomegalievirusausscheidung bei gesunden Kindern im ersten Lebensjahr *Schweiz Med Wochenschr 104* 468 1974
17 Taran A A simple method for performing a Wassermann test on anticomplementary serum *J Lab Clin Med 31* 1037 1946

Submitted July 11 1978
Accepted Jan 25 1979

(K A) Department of Clinical Virology
Malmo General Hospital
21401 Malmo
Sweden

Acta Pædiatr Scand 68 513–519 1979

CLINICAL FINDINGS AND INTESTINAL IMMUNOGLOBULINS IN CHILDREN WITH PARTIAL IgA DEFICIENCY

E SAVILAHTI and P PELKONEN

From the Childre ı s Hospital Unıı ersıt\ of Helsınkı Helsınkı Fınland

ABSTRACT Savılahtı E and Pelkonen P (Children s Hospıtal University of Helsinkı Helsinkı Fınland) Clınıcal findings and ıntestinal ımmunoglobulıns ın children with partıal IgA deficıency Acta Paedıatr Scand 68 513 1979 —We studied the intestınal morphology and the jejunal and rectal ımmunoglobulıns of 16 children with partıal IgA deficiency defined as serum IgA concentration more than two standard deviations below the mean for age but higher than the lower lımıt of sensitivity of single radial ımmunodiffusion (0 02 g/l) Five of the patients had been treated with phenytoın 2 had juvenıle rheumatoıd arthrıtıs 2 had ulcerative colıtıs and 5 had recurrent upper respiratory tract ınfections The jejunal morphology was normal in every case In 6 cases normalization of serum IgA occurred durıng the follow up while ın one patient with ulcerative colıtıs the concentration fell below 0 02 g/l In patients with recurrent ınfections there was a decreased frequency of infections when the level of serum ıgA ıncreased In 4 patients IgM-containıng cells predominated ın both the jejunal and rectal mucosa and IgM was ıncreased ın the intestinal juice In 6 patients a sıgnıficant ıncrease ın IgM containıng cells or a decrease ın IgA containıng cells or both were seen ın either the rectal or jejunal mucosa There was no correlation between the number of IgA containıng cells ın the ıntestınal mucosa and the serum level of IgA

KEY WORDS Partıal IgA deficıency low serum IgA ıntestınal ımmunoglobulıns

Selective deficıency of IgA ıs a well-characterızed and common ımmunodeficıency (2 8 12 21) In addıtıon to the deficıency of IgA ın serum and secretıons there ıs replacement of IgA by IgM ın the mucous membranes and secretıons (5 8 23) The clınıcal features of IgA deficıency are well known Much less ıs known about the ımportance of partıal IgA deficıency ıts clınıcal assocıatıons and mucosal ımmunoglobulıns In recent years there have been clınıcal reports of patıents wıth a decrease ın serum IgA attrıbuted to treatment wıth phenytoın (1 3 24) and *d* penıcıllamıne (10) These patıents have had low but measurable quantıtıes of serum IgA Thıs report ıs based on our experıence wıth chıldren havıng a partıal deficıency of serum IgA In addıtıon to clınıcal characterıstıcs we have studıed mucosal ımmunoglobulıns ın the ıntestıne of 16 such patıents Most of them have been followed for several years and changes ın the serum and rectal ımmunoglobulıns have been documented

MATERIALS

The study ıncludes 16 chıldren over years of age whose serum level of IgA was more than two SD below the mean for age but above the lower lımıt of sensıtıvıty of sıngle radıal ımmunodıffusıon (0 0? g/l) (Fıg 1) They had normal or elevated levels of IgG IgM and IgE The patıents were detected among 3 000 chıldren over 2 years of age whose sera were sent to routıne ımmunoelectrophoretıc study ın 1972–73 ın the Chıldren s Hospıtal Unıversıty of Helsınkı Durıng the same perıod 12 subjects wıth selectıve IgA deficıency (serum concentratıon below 0 00 g/l) were found

The results on the ıntestınal ımmunoglobulıns are compared wıth those of ımmunologıcally normal chıldren (?) and chıldren wıth selectıve IgA deficıency (3) studıed by the same method

METHODS

Serum concentratıons of IgA IgG and IgM were measured by the sıngle radıal ımmunodıffusıon method and compared wıth normal values of chıldren establıshed ın

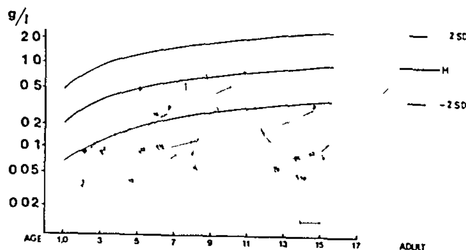


Fig 1 Serum IgA levels of the patients Mean (M) +2 S D and −2 S D for age are indicated (II) The time of the intestinal studies and of the follow up rectal biopsies is given (/) The case numbers correspond to those in Table 1

the same laboratory (11) The antisera of IgA IgG and IgM used were products of Hyland Laboratories Our serum standard has been originally standardized using a serum sample which had been assayed for the immunoglobulin concentrations in the laboratory of Dr D Gitlin Pittsburgh Pa The serum standard has subsequently been calibrated against the WHO reference preparation 67/97 (18) and the transformation coefficients are IgA 1 IU=0 88 mg IgM 1 IU=0 60 mg and IgG 1 IU=9 1 mg IgE was measured with the commercial radioimmunoabsorbent kit Phadebas® (Pharmacia Sweden) Antibodies to cow s milk and gluten were studied by a double diffusion micromethod (11) Intestinal juice was collected via biopsy tubing Its content of IgA IgM and IgG was measured by electroimmunodiffusion (22)

Small intestinal biopsy specimens were taken from the upper jejunum with a Crosby Kugler biopsy capsule The specimens were studied under a dissecting microscope and divided for light microscopy and immunofluorescense study in addition a part of 11 biopsy specimens was used for the measurements of maltase sucrase lactase and isomaltase activities (20) The morphology was examined as described by Kuitunen (13) Tissue for the immunofluorescence study was processed and stained with FITC conjugated anti IgA IgM IgG IgE and IgD antisera as described earlier (2?) The number of positively stained cells in an area of at least 0 2 mm² between surface epithelium and muscularis mucosae was counted directly on the slides at 1000 fold magnification under a Leitz microscope with epi illumination A mercury vapour lamp was used as the light source with KR 490 interference exciter filter and TK 495 and K 495 and K 530 barrier filters (17)

RESULTS

Clinical findings and follow up of IgA levels
The intestinal studies were performed at or shortly after the time the partial IgA deficiency was detected (Fig 1) Patients 1–5 (Table 1) had received phenytoin treatment prior to the finding of a low IgA value In patients 2 4 and 5 a normal serum IgA had been documented before phenytoin treatment was started (Fig 1) In cases 1–3 a control sample taken after discontinuation of the treatment showed an elevation of the IgA level but it was still more than 2 S D below the mean for age in case 3 In case 4 there was some increase in IgA in spite of continued phenytoin treatment In the two patients with ulcerative colitis (cases 8 and 9) a normal IgA was recorded in the early phase of the disease (Fig 1) Both were treated with Salazopyrin® The older child had also chronic active hepatitis and received at times a small dose of prednisone His IgA level fell to a very low value (below 0 02 g/l) and remained undetectable In both cases with juvenile rheumatoid arthritis (JRA) we saw normalization of serum IgA during the follow up period (Table 1) Three children had atopic disease and frequent respiratory tract infections All had been treated with anticongestants prior to the finding of low IgA (Table 1) In case 12 a normal IgA was recorded before commencement of medication

Levels of IgG IgM and IgE One patient had an IgG level more than 2 S D below the mean for age at the initial examination but it rose during the follow up (case 16 in Table 1) In patient 7 with JRA increased concentrations of IgG were observed The IgM levels of the patients were within the normal range Two patients had elevated levels of IgE and both of them had atopic symptoms

Table 1 *Clinical and immunological data*

Case	Age at examination (years)	Clinical diagnosis	Medication (age period)	Clinical state at follow up	Serum immunoglobulins (g/l) IgA	IgG	IgM	Precipitating antibodies to cow s milk
1	6 6	RURTI febrile convulsions	Phenytoin (0 ?–4 0)	Improved	0 03 0 26	6 5 11 1	0 34 0 41	– –
?	3 1 7 7	RURTI febrile convulsions	Phenytoin (1 7–4 5)	Improved	0 08 0 80	9 ? 13 4	1 32 0 71	– –
3	14 5 17 7	Abdominal pains	Phenytoin (9 5–14 6)	Asymptomatic	0 08 0 6	10 0 8 5	0 9? 0 56	+ +
4	10 4 14 4	RURTI febrile convulsions	Phenytoin (9 5–cont)	Improved	0 1? 0 30	1? 4 1? 0	0 48 0 59	+ +
5	13 6	Convulsions	Phenytoin (10 3–cont)	–	0 08	10 5	1 40	–
6	2 ? 5 0	*Rheumatoid* arthritis	Chloroquine (1 5–cont) Salicylates (3 ?–3 7)	Inactive JRA	0 06 0 43	6 4 1? 6	0 05 1 00	– –
7	15 0 18 7	Rheumatoid arthritis	Chloroquine (9 5–1? 1) Myochrysine (11 8–cont)	Active JRA	0 09 0 66	?8 7 36 6	1 30 1 45	+ +
8	8 10 7	Ulcerative colitis	Salazopyrin (7 5–cont)	Asymptomatic	0 13 0 ?8	14 ? 11 7	0 56 0 41	– –
9	1 5 15 5	Ulcerative colitis chr active hepatitis	Prednison (10–14) Salazopyrin (10 7–14 0)	Asymptomatic	0 05 <0 0?	7 1 29 3	0 40 1 42	+ +
10	13 7 16 9	Diabetes mellitus *thyroiditis*	Insulin (1? 1–cont) *Thyroxin (1 6–cont)*	Unchanged	0 05 0 33	1 1 13 1	0 55 0 51	– –
11	14 3	Acute nephritis	–	–	0 14	13 0	0 97	–
1?	4 7 7 1	RURTI	Anticongestant (4 5–cont)	Improved	0 03 0 08	7 5 9	0 56 0 37	+ +
13	5 4 9 4	Allergic rhinitis RURTI	Anticongestant (3–6)	Improved	0 08 0 32	10 2 12 6	0 68 0 38	– –
14	8 4	Bronchial asthma	Anticongestant (?–8 4)	–	0 06	10 1	0 52	–
15	5 9 9 9	Dystonic tetraplegia athethosis	Diazepam (? 1–cont)	Improved	0 ?? 0 6?	15 4 13 4	0 70 0 69	+ +
16	6 3 8 1	Urinary tract infections	Sulfisoxazole (6 3–8 0)	Asymptomatic	0 11 0 1?	5 7 7 5	0 50 0 44	– –

RURTI=recurrent upper respiratory tract infections Anticongestant' contains phenylephrine chlorpheniramine and mepyramine Anticongestant contains phenylpropanolamine and cinnarizine

Serum antibody studies Antibodies to gluten were not found in any of the cases but six of the patients had precipitating antibodies to cow s milk (Table 1) Four of the six had abnormal cell counts in their jejunal mucosa (Fig 2) one had a tendency to tracheal aspiration on account of his neurological disease

Intestinal morphology and disaccharidase activities The morphology of the jejunal biopsy specimens was normal in all 16 cases In one patient lactase deficiency was found (lactase activity 6 U/g patient 3 normal values >20 U/g) He had been treated with phenytoin because of suspected abdominal epilepsy Normal disaccharidase activities were found in the other 10 cases studied Patient 8 with ulcerative colitis had inflammatory changes in the rectal biopsy specimen all the other rectal biopsies were morphologically normal

Immunoglobulin containing cells in intestinal mucosa The total numbers of immunoglobulin-containing cells were very similar in immunologically normal children patients

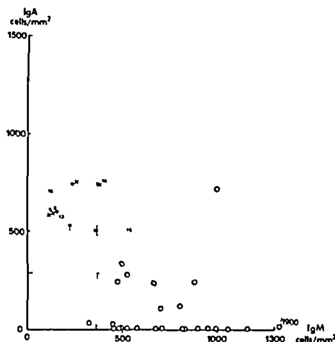


Fig. 2. Numbers of IgA- and IgM-containing cells in the jejunal mucosa of patients (× and case numbers as in Table 1), immunologically normal children (●) and children with selective IgA deficiency (O). The mean −2 S.D. for IgA and the mean +2 S.D. for IgM cell numbers of the normal children are indicated.

with low serum IgA and patients with selective IgA deficiency (Table 2). The number of IgA- and IgM-containing cells in the individual cases in this study, in children with normal immunoglobulins and in patients with selective IgA deficiency are given for jejunal mucosa in Fig. 2 and for rectal mucosa in Fig. 3. In 14 biopsy specimens of 10 patients the number and ratio of IgA- and IgM-containing cells was abnormal, defined as a decrease of IgA-containing cells to a number less than the mean −2 S.D. of normals, an increase of IgM cells to a number more than the mean +2 S.D. of normals, or the presence of more IgM than IgA cells. Four patients had abnormal results in both jejunal and rectal biopsies (cases 7, 9, 12, 14); in 6 patients the cell count was abnormal in one of these tissues and 6 patients had findings within 2 standard deviations of the controls. No correlation between the serum IgA level and the number of IgA-containing cells in the intestinal mucosa could be found. In individual cases the alteration in the mucosal immunoglobulins was unpredictable, e.g. the patient with the highest serum IgA (case 15) had an abnormal cell count in the jejunal mucosa, and the finding in patient 10 with a low serum IgA value (0.05 g/l) was normal in both biopsy specimens.

The patients had significantly more IgG-containing cells in both the rectal ($p<0.05$) and the jejunal mucosa ($p<0.01$) than immunologically normal children (Table 2) and also more than patients with selective IgA deficiency. IgE- and IgD-containing cells were equally rare in the biopsy specimens from both groups of patients and from normal children.

In five patients a rectal biopsy specimen was studied in connection with the follow-up (Fig. 1). All had normal numbers of immunoglobulin-containing cells. In two (cases 1 and 3) the finding had been abnormal in the earlier biopsy specimen.

Immunoglobulins in the intestinal juice. On the average, patients with low serum IgA had

Table 2. *Total numbers of immunoglobulin-containing cells and IgG-containing cells in the intestinal mucosa*

	No. of cases	Jejunal mucosa, cells/mm² Mean±S.D. Total	IgG	No. of cases	Rectal mucosa, cells/mm² Mean±S.D. Total	IgG
Patients with partial IgA deficiency	16	970±176	101±61	15	609±231	103±81
Children with normal serum immunoglobulins	17	1 030±331	53±26	12	619±188	20±17
Patients with selective IgA deficiency	25	931±379	83±51	24	448±245	36±35

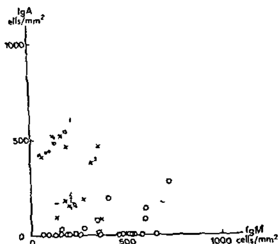


Fig 3 Numbers of IgA and IgM-containing cells in the rectal mucosa Symbols as in Fig 2

only slightly more IgM and less IgA in the intestinal juice than children with normal immunoglobulins (Table 3) The concentration of IgM was higher than that of IgA in 8 samples from 15 patients in only one of these was the number of IgA and IgM containing cells normal in the biopsy specimens This ratio in the intestinal juice was seen in 3 out of 15 children with normal serum immunoglobulins

DISCUSSION

It is well established that immunologically normal persons have a predominance of IgA containing cells *in their intestinal* mucosa (7 19 22) In selective IgA deficiency this abundant IgA cell population is replaced by IgM containing cells (8 23) However in a study of a large group of IgA deficient subjects some of them were found to have significant numbers of IgA-containing mucosal cells and these patients also had some IgA in their sera (23) In the present series of patients with partial IgA deficiency we found that they had normal total numbers of immunoglobulin-containing cells and 6 of them had normal ratios of IgA and IgM-containing cells However 10 patients showed either an increase in IgM containing cells or a decrease of IgA-containing cells or both This change from the normal cell ratio varied in degree and in some of the patients it was evident only in either the jejunal or the rectal specimens whereas in others the finding was similar to that found in selective IgA deficiency No correlation between the serum level of IgA and the cell numbers could be found With regard to mucosal immunoglobulin production patients with partial IgA deficiency form a transitional group between persons *with normal serum* immunoglobulins and patients with selective IgA deficiency the mucosal immunoglobulins are unpredictable on the basis of the serum level of an individual patient The situation is compatible with *a varying degree of suppression* of IgA production by T cells (25) resulting in disproportion in serum level of IgA and the number of IgA containing cells in the intestine Similar dichotomy between serum and gut immunoglobulins has been described in patients with *hypogammaglobulinaemia* (4)

A greater number of IgG-containing cells were noted in the mucosal samples than in earlier group studies (22 23) The difference may be *partly explained* by technical differences since during this study a change was made to epi illumination and this facilitated the dis

Table 3 *Immunoglobulins in the intestinal juice*

	No of samples	IgA g/l (mean±S D)	IgM g/l (mean±S D)
Patients with partial IgA deficiency	15	0 073±0 017	0 025±0 015
Children with normal serum immunoglobulins	15	0 047±0 018	0 023±0 017
Patients with selective IgA deficiency	24	Not calculated	0 080±0 041

tinction of IgG-containing cells from the background. Specimens of 14 patients in this study were measured with epi-illumination.

The association of low serum IgA and phenytoin treatment has been previously verified (1, 3, 24). Five of our patients were on phenytoin at the time when the low serum IgA was noted. A normal pretreatment level of serum IgA was found in three cases and normalization of the level occurred after discontinuation of the drug in three other cases. In addition to phenytoin, *d*-penicillamine has been shown to depress serum IgA (10). On the basis of our study, another group of drugs can be suspected of exerting a similar effect, namely anticongestants containing antihistamines and vasoconstrictive agents. Three of the patients were receiving these drugs, all on a long term basis. In one we found a normal IgA level prior to anticongestant therapy and in another normalization of IgA level took place after discontinuation of the treatment.

In the course of this study, we found two patients with low serum IgA and JRA. In an analysis of a large group of children with JRA, 19 cases with decreased and also deficient serum IgA were discovered (16), 9 of which turned out to be transient. A transient depression of IgA was seen in one of two patients with ulcerative colitis (UC), while in the other case IgA gradually fell and total IgA deficiency developed. A similar decrease of IgA has been previously described in a patient with chronic active hepatitis (14) and in one case of monozygotic twins after an intestinal infection (15). The cases with JRA and UC in this study has been treated with multiple drugs and one may speculate whether the changes in serum IgA are due to drug treatment or due in part to the primary disease.

Our series includes four patients presenting with recurrent upper respiratory tract infections and two with congenital neurological disease. It is possible that some of these patients have a genetically determined alteration in the production of IgA, since two patients have a stable low level of IgA.

In at least 5 patients low serum IgA was documented during a period of frequent respiratory infections. In every one of these cases infections became more rare as serum IgA increased, suggesting the importance of secretory IgA in the defence of mucosal membranes. Although many persons with selective IgA deficiency are in good health (12), respiratory infections are the presenting symptom in a large number of cases with this deficiency (2, 21). Local immunoglobulins in the gut are important in protection against absorption of ingested native proteins from the intestine of rats (26). In man this antigen exclusion is probably provided by secretory IgA (26), e.g. human milk has secretory IgA antibodies to cow's milk proteins, which are capable of reducing antibody response to cow's milk in infants (9). As a sign of defective antigen exclusion most persons with IgA deficiency have precipitating antibodies to cow's milk proteins (6, 21); in this group they were also more common than in controls and their presence was associated in most cases with a decrease in jejunal IgA containing cells. The frequency of coeliac disease is much increased in selective IgA deficiency (21) and up to date we have found 7 children with coeliac disease out of 75 with a selective IgA deficiency in whom jejunal biopsy had been performed. In children with partial IgA deficiency we did not find a single case with abnormal jejunal mucosa; therefore we have come to the conclusion that performing an intestinal biopsy on asymptomatic children with low serum IgA is not indicated.

We feel that it is of clinical importance to make a clear distinction between partial and total IgA deficiency. Partial IgA deficiency is in most cases a transient phenomenon followed by an increase in IgA, though in one of our cases an acquired total deficiency of IgA resulted. Partial IgA deficiency is often seen in connection with various drug regimens and in that case discontinuation of the drug should be considered. In patients with partial IgA deficiency, mucosal immunoglobulins can

belong predominantly to either the IgA or the IgM class

ACKNOWLEDGEMENTS

This study was supported by grants from the Finnish Medical Foundation and the Foundation for Paediatric Research Finland

REFERENCES

1. Aarli J A & Tonder O Effect of antiepileptic drugs on serum and salivary IgA *Scand J Immunol 4* 391 1975
2. Ammann A J & Hong R Selective IgA deficiency Presentation of 30 cases and a review of the literature *Medicine 50* 2 3 1971
3. Andersen P & Mosekilde L Immunoglobulin levels and autoantibodies in epileptics on long term anticonvulsant therapy *Acta Med Scand 210* 69 1977
4. Broom B C de la Concha E G Webster A D B Loewi G & Asherson G L Dichotomy between immunoglobulin synthesis by cells in gut and blood of patients with hypogammaglobulinaemia *Lancet II* 53 1975
5. Brandtzaeg P Fjellander I & Gjeruldsen S T Immunoglobulin M Local synthesis and selective secretion in patients with immunoglobulin A deficiency *Science 160* 789 1968
6. Buckley R H & Dees S C Correlation of milk precipitins with IgA deficiency *N Engl J Med 281* 465 1969
7. Crabbé P A Carbonara A O & Heremans J F The normal human intestinal mucosa as a major source of plasma cells containing γA immunoglobulin *Lab Invest 14* 35 1965
8. Crabbé P A & Heremans J F Lack of gamma A-immunoglobulin in serum of patients with steatorrhoea *Gut 7* 119 1966
9. Hanson L Å Ahlstedt S Carlsson B & Fallstrom S Secretory IgA antibodies against cow s milk proteins in human milk and their possible effect in mixed feeding *Int Arch Allergy Appl Immun 1 54* 457 1977
10. Hjalmarson O Hanson L Å & Nilsson L Å IgA deficiency during D-penicillamine treatment *B Med J I* 549 1977
11. Immonen P Levels of serum immunoglobulins γA γG and γM in the malabsorption syndrome in children *Ann P ie l at Fenn 13* 115 1967
12. Koistinen J Selective IgA deficiency in blood donors *Vox Sang 29* 1 1975
13. Kuitunen P Duodeno-jejunal histology in malabsorption syndrome in infants *Ann Paediatr Fenn 12* 101 1966
14. Lawton A R Wu L Y E & Cooper M D The cellular basis of IgA deficiency in human *In* J Mestecky & A R Lawton (eds) *The Immunoglobulin A system* Plenum Press New York and London 1974 p 373
15. Lewkonia R M Gairdner D & Doe W F IgA deficiency in one of identical twins *Br Med J I* 311 1976
16. Pelkonen P Savilahti E Westeren L & Makela A L IgA deficiency in juvenile rheumatoid arthritis *Scand J Rheumatol* Suppl 8 013 1975
17. Ploem J S The use of a vertical illuminator with interchangeable dichroic mirrors for fluorescence microscopy with incident light *Z Wiss Mikr 68* 1 9 1967
18. Rowe D S Anderson S G & Grab B A research standard for human serum immunoglobulin IgG IgA and IgM *Bull WHO 42* 535 1970
19. Rubin W Fauci A S Sleisenger M H & Jeffries G H Immunofluorescent studies in adult celiac disease *J Clin Invest 44* 475 1965
20. Sahi T Isokoski M Jussila J & Launiala K Lactose malabsorption in Finnish children of school age *Acta Paediatr Scand 61* 11 1971
21. Savilahti E Pelkonen P & Visakorpi J K IgA deficiency in children A clinical study with special reference to intestinal findings *Arch Dis Child 46* 665 1971
22. Savilahti E Immunoglobulin-containing cells in the intestinal mucosa and immunoglobulins in the intestinal juice in children *Clin Exp Immunol 11* 415 1972
23. Savilahti E IgA deficiency in children Immunoglobulin-containing cells in the intestinal mucosa immunoglobulins in secretions and serum IgA levels *Clin Exp Immunol 13* 395 1973
24. Seager J Jamison D I Wilson J Hayward A R & Soothill J F IgA deficiency epilepsy and phenytoin treatment *Lancet III* 632 1975
25. Waldmann T A Durm M Broder S Blackman M Blease R M & Strober W Role of suppressor T cells in pathogenesis of common variable hypogammaglobulinaemia *Lancet I* 609 1974
26. Walker W A Wu M Isselbacher K J & Bloch K J Intestinal uptake of macromolecules III Studies on the mechanism by which immunization interferes with antigen uptake *J Immunol 115* 854 1975

Submitted Sept 6 1978
Accepted Nov 13 1978

(E S) Children s Hospital
00290 Helsinki 29
Finland

tinction of IgG-containing cells from the background Specimens of 14 patients in this study were measured with epi illumination

The association of low serum IgA and phenytoin treatment has been previously verified (1 3 24) Five of our patients were on phenytoin at the time when the low serum IgA was noted A normal pretreatment level of serum IgA was found in three cases and normalization of the level occurred after discontinuation of the drug in three other cases In addition to phenytoin *d* penicillamine has been shown to depress serum IgA (10) On the basis of our study another group of drugs can be suspected of exerting a similar effect namely anticongestants containing antihistamines and vasoconstrictive agents Three of the patients were receiving these drugs all on a long term basis In one we found a normal IgA level prior to anticongestant therapy and in another normalization of IgA level took place after discontinuation of the treatment

In the course of this study we found two patients with low serum IgA and JRA In an analysis of a large group of children with JRA 19 cases with decreased and also deficient serum IgA were discovered (16) 9 of which turned out to be transient A transient depression of IgA was seen in one of two patients with ulcerative colitis (UC) while in the other case IgA gradually fell and total IgA deficiency developed A similar decrease of IgA has been previously described in a patient with chronic active hepatitis (14) and in one case of monozygotic twins after an intestinal infection (15) The cases with JRA and UC in this study has been treated with multiple drugs and one may speculate whether the changes in serum IgA are due to drug treatment or due in part to the primary disease

Our series includes four patients presenting with recurrent upper respiratory tract infections and two with congenital neurological disease It is possible that some of these patients have a genetically determined alteration in the production of IgA since two patients have a stable low level of IgA

In at least 5 patients low serum IgA was documented during a period of frequent respiratory infections In every one of these cases infections became more rare as serum IgA increased suggesting the importance of secretory IgA in the defence of mucosal membranes Although many persons with selective IgA deficiency are in good health (12) respiratory infections are the presenting symptom in a large number of cases with this deficiency (2 21) Local immunoglobulins in the gut are important in protection against absorption of ingested native proteins from the intestine of rats (26) In man this antigen exclusion is probably provided by secretory IgA (26) e g human milk has secretory IgA antibodies to cow s milk proteins which are capable of reducing antibody response to cow s milk in infants (9) As a sign of defective antigen exclusion most persons with IgA deficiency have precipitating antibodies to cow s milk proteins (6 21) in this group they were also more common than in controls and their presence was associated in most cases with a decrease in jejunal IgA containing cells The frequency of coeliac disease is much increased in selective IgA deficiency (21) and up to date we have found 7 children with coeliac disease out of 75 with a selective IgA deficiency in whom jejunal biopsy had been performed In children with partial IgA deficiency we did not find a single case with abnormal jejunal mucosa therefore we have come to the conclusion that performing an intestinal biopsy on asymptomatic children with low serum IgA is not indicated

We feel that it is of clinical importance to make a clear distinction between partial and total IgA deficiency Partial IgA deficiency is in most cases a transient phenomenon followed by an increase in IgA though in one of our cases an acquired total deficiency of IgA resulted Partial IgA deficiency is often seen in connection with various drug regimens and in that case discontinuation of the drug should be considered In patients with partial IgA deficiency mucosal immunoglobulins can

belong predominantly to either the IgA or the IgM class

ACKNOWLEDGEMENTS

This study was supported by grants from the Finnish Medi al Foundation and the Foundation for Paediatri Research Finland

REFERENCES

1 Aarli J A & Tonder O Effect of antiepileptic drugs on serum and salivary IgA *Scand J Immunol 4* 391 1975

2 Ammann A J & Hong R Selective IgA deficiency Presentation of 30 cases and a review of the litera ture *Medicine 50* 223 1971

3 Andersen P & Mosekilde L Immunoglobulin levels and autoantibodies in epileptics on long term anticonvulsant therapy *Acta Med Scand 210* 69 1977

4 Broom B C de la Concha E G Webster A D B Loewi G & Asherson G L Dichotomy be tween immunoglobulin synthesis by cells in gut and blood of patients with hypogammaglobulinaemia *Lancet II* 53 1975

5 Brandtzaeg P Fjellander I & Gjeruldsen S T Immunoglobulin M Local synthesis and selective secretion in patients with immunoglobulin A defi ciency *Science 160* 789 1968

6 Buckley R H & Dees S C Correlation of milk precipitins with IgA deficiency *N Engl J Med 281* 465 1969

7 Crabbe P A Carbonara A O & Heremans J F The normal human intestinal mucosa as a major source of plasma cells containing γA immunoglobu lin *Lab Invest 14* 35 1965

8 Crabbé P A & Heremans J F Lack of gamma A immunoglobulin in serum of patients with steator rhoea *Gut 7* 119 1966

9 Hanson L Å Ahlstedt S Carlsson B & Fall strom S Secretory IgA antibodies against cow s milk proteins in human milk and their possible ef fect in mixed feeding *Int A ch Allergy Appl Im munol* 54 457 1977

10 Hjalmarson O Hanson L Å & Nilsson L Å IgA defi iency during D-penicillamine treatment *Br Med J I* 549 1977

11 Immonen P Levels of serum immunoglobulins γA γG and γM in the malabsorption syndrome in chil dren *Ann Paedi tr Fenn 13* 115 1967

12 Koi tinen J Selective IgA deficiency in blood donors V r S ing 9 1 1975

13 Kuitunen P Duodeno-jejunal histology in malab sorption syndrome in infants *Ann Paediatr Fenn 12* 101 1966

14 Lawton A R Wu L Y E & Cooper M D The cellular basis of IgA deficiency in human *In* J Me stecky & A R Lawton (eds) *The Immunoglobulin A system* Plenum Press New York and London 1974 p 373

15 Lewkonia R M Gairdner D & Doe W F IgA deficiency in one of identical twins *Br Med J I* 311 1976

16 Pelkonen P Savilahti E Westeren L & Makela A L IgA deficiency in juvenile rheumatoid arthritis *Scand J Rheumatol* Suppl 8 013 1975

17 Ploem J S The use of a vertical illuminator with interchangeable dichroic mirrors for fluorescence microscopy with incident light *Z Wiss Mikr 68* 129 1967

18 Rowe D S Anderson S G & Grab B A re search standard for human serum immunoglobulin IgG IgA and IgM *Bull WHO 42* 535 1970

19 Rubin W Fauci A S Sleisenger M H & Jeffries G H Immunofluorescent studies in adult celiac dis ease *J Clin Invest 44* 475 1965

20 Sahi T Isokoski M Jussila J & Launiala K Lactose malabsorption in Finnish children of school age *Acta Paediatr Scand 61* 11 1971

21 Savilahti E Pelkonen P & Visakorpi J K IgA deficiency in children A clinical study with special reference to intestinal findings *Arch Dis Child 46* 665 1971

22 Savilahti E Immunoglobulin-containing cells in the intestinal mucosa and immunoglobulins in the in testinal juice in children *Clin Exp Immunol 11* 415 1972

23 Savilahti E IgA deficiency in children Immuno globulin-containing cells in the intestinal mucosa im munoglobulins in secretions and serum IgA levels *Clin Exp Immunol 13* 395 1973

24 Seager J Jamison D I Wilson J Hayward A R & Soothill J F IgA deficiency epilepsy and pheny toin treatment *Lancet III* 632 1975

25 Waldmann T A Durm M Broder S Blackman M Blease R M & Strober W Role of suppressor T cells in pathogenesis of common variable hypo gammaglobulinaemia *Lancet I* 609 1974

26 Walker W A Wu M Isselbacher K J & Bloch K J Intestinal uptake of macromolecules III Studies on the mechanism by which immunization in terferes with antigen uptake *J Immunol 115* 854 1975

Submitted Sept 6 1978
Accepted Nov 13 1978

(E S) Children s Hospital
00290 Helsinki 29
Finland

A PROSPECTIVE STUDY OF INDIVIDUAL COURSES OF BREAST FEEDING

STIG SJÖLIN YNGVE HOFVANDER and CHARLOTTE HILLERVIK

From the Department of Paediatrics University Hospital Uppsala Sweden

ABSTRACT Sjolin S Hofvander Y and Hillervik C (Department of Paediatrics University Hospital Uppsala Sweden) A prospective study of individual courses of breast feeding Acta Paediatr Scand 68 521 1979 —A prospective study of the course of breast feeding was carried out in 75 randomly selected women Weekly interviews were performed from the day after delivery until the termination of breast feeding but in no case for longer than 6 months In each case a detailed analysis was made of the factors leading to transient lactation crises or to complete cessation of breast feeding A second group of 71 mothers also randomly selected was interviewed in retrospect only 6 months after delivery and served as controls Twenty four weeks after delivery 47% of the mothers in the weekly interview group were still breast feeding The corresponding figure in the control group was 38% In both groups only few mothers terminated lactation for medical reasons while about one fourth stopped for some other reason and about one half because of a combination of factors Brief case reports are presented to illustrate how varying the factors were that threatened breast feeding

KEY WORDS Breast feeding lactation crise

In a previous retrospective study we made an attempt to analyse the causes of early termination of breast feeding (1) Some general characteristics of those mothers who breast fed for more than 8 weeks were discerned In many cases it was also possible to record a reason for the early weaning Eventually however we reached the conclusion that the direct and real cause of early weaning was not revealed by this retrospective epidemiological method We therefore decided to apply quite a different approach by following the course of breast feeding continuously and intensely in a limited number of mothers from delivery until the child was weaned or 6 months old By this means we expected to obtain more detailed and more precise information about the direct causes of termination or threatened termination of breast feeding In order to estimate the interviewer s influence on the course of breast feeding a control group of mothers were interviewed in retrospect 6 months after delivery

MATERIAL

During the period November 1973–November 1974 78 women were selected at random at the Maternity Department of the University Hospital in Uppsala Sweden Each week the two mothers who were the last to be delivered on Monday (before midnight) were selected Only healthy mothers resident in Uppsala with healthy babies normally delivered and weighing more than 3 kg were included These mothers are henceforth referred to as the *interview group* For practical reasons the study could not be conducted continuously but had to be interrupted during part of the summer and during long holidays

The two women who were delivered immediately before those in the interview group on a Monday were selected for assignment to a *control group* with the same criteria for inclusion This group also comprised 78 women

Three mothers from the interview group refused to participate after having left the maternity ward Seven mothers in the control group moved from Uppsala and were thus excluded Thus there remained 75 mothers in the *interview group* and 71 in the *control group*

Table 1 *Duration of breast feeding*

) On discharge from the maternity ward

	Interview group		Control group	
Time weeks	Completely breast fed (%)	Completely and partially breast fed (%)	Completely breast fed (%)	Completely and partially breast fed (%)
0	95	97	94	99
2	89	95	93	96
4	85	92	79	87
8	77	83	69	75
12	63	79	52	66
16	51	68	39	52
20	36	60	28	45
24	17	47	15	38

Partially means that cow s milk formulas were given together with breast milk

this duration was somewhat longer in the *interview group* and further that extremely few babies left the maternity ward solely on a diet of breast milk substitutes

B *Transient lactation crises in the interview group*

During the course of lactation 46 of the 75 mothers ran into breast feeding difficulties on a total of 69 occasions (Table 2) Seventeen mothers had more than one episode of this kind The crises could be recorded and analysed through the weekly contacts When the mother herself contacted the interviewer she was usually in immediate need of help and advice In most cases there were obvious and often trivial causes resulting in a decreased milk output For example the mother was perhaps temporarily tired or the child was crying for unusually long periods for no obvious reason Sometimes however more worrying problems either alone or as part of a chain of events caused a temporary reduction of the milk output (Table 2) It appeared and this is of fundamental importance that each *individual* mother reacted in her own special way to motherhood and to difficult situations Certain mothers enjoyed their maternal role and were able to quietly endure the child s crying night feeds sore nipples etc while other mothers were tied to and constantly bothered about weight curves grams time schedules the child s crying and frequent breast feeds

The number and percentage of different reasons for transient lactation crises in the interview group are presented in Table 3 Emotional disturbances in the mother (39%) and problems concerning the child (32%) were predominant Most of the crises occurred during the first months—52% before two months and 77% before three By general support and simple advice it was often possible to postpone weaning for a considerable time In 53 instances out of 69 final cessation of breast feeding was delayed by 4 weeks or more and in 25 cases by 12 weeks or more

C *Reasons for curtailed breast feeding*

Table 3 shows how the reasons for curtailment of breast feeding in the interview and control groups were distributed Reasons emanating from the interplay between the mother and the environment and rational reasons were the most common in both groups It is striking that emotional reasons were more common in the interview group than in the *retrospectively* interviewed control group

A brief review of the reasons or events

The two groups were very similar with respect to age civil status education and type of occupation There was a high percentage of mothers who were studying or had completed studies at a university or college (28% in both groups) About half of the mothers were primiparas

METHODS

The interview group

Interviews were conducted regularly starting at the maternity ward on day 1 (recruiting interview) and then continuing on day 4 whereafter home visits were made when the infant was 2 weeks 6 weeks and 3 months old In addition the mothers were interviewed by telephone once weekly throughout the period of breast feeding Furthermore they were encouraged to contact the interviewer at once if the breast feeding was disturbed or threatened or if they felt in need of support and advice A final interview was held after 6 months with those who were still breast feeding at that time

During these interviews a standardized questionnaire with pre coded as well as open-ended questions was used [1]

The interviews held at home after 2 weeks 6 weeks and 3 months were designed to provide information not only on the child s feeding but also on the mothers s physical condition her attitude to the breast feeding and her attitude to the child and to her husband and further on the extent to which she was helped with the domestic work

The weekly interviews by telephone included questions as to whether the mother was breast feeding wholly or partially or whether she had discontinued or intended to discontinue breast feeding If relevant she was asked what kind and amount of breast milk replacer she was giving If the mother was intending to stop breast feeding or if involuntary discontinuation was imminent (hereafter referred to as a lactation crisis) a more detailed analysis was made concerning the cause mainly with open ended questions Immediate home visits were often made in such cases

During the study the interviewer thus had contact with each mother a large number of times and was able to follow any events or chain of events which threatened to terminate the breast feeding

Particularly when the mother had decided to stop breast feeding or when a lactation crisis was imminent attempts were made during the interviews to find out and to evaluate the *real* cause or motive or the starting point in a chain of events

The control group

Contact was made with these mothers 6 months after delivery Before that time they were not aware that they had been selected for the study Apart from data concerning their socioeconomic situation and family background the interview included analysis with regard to the time of weaning and reasons why the mother had started to wean the child or had stopped breast feeding completely A standardized questionnaire with pre coded and open ended questions was used

All interviews in both groups were conducted by one of the authors (C H) All the mothers co-operated well

Classification of reasons for transient lactation crises and curtailed breast feeding

Successful breast feeding requires harmonious interplay between nervous and hormonal impulses within the mother between the mother and the child and between the mother and the environment at large Any of these relationships may be disturbed and influence the course of breast feeding During our analyses and evaluations of the numerous reasons for lactation crises or termination of breast feeding that were revealed at the interviews it became obvious that the best and clearest way to present these reasons was to classify them according to the scheme laid out below In many cases there were several underlying factors but only in a few cases was there any doubt as to which was the *main reason* for the lactation crisis or curtailed breast feeding Thus it was generally easy to decide to which group a mother should be assigned For obvious reasons the case histories presented in Tables 2 and 4 are only brief and do not tell the whole story but nevertheless they give essential information about the causes of threatened breast feeding in these mothers

1 Reasons connected with *the mother*

(*a*) Medical e g breast complications general illness lactation psychosis

(*b*) Rational e g deliberate weaning usually due to gainful employment outside the home or studies or because the mother considered that she had breast fed long enough

(*c*) Emotional e g unhappiness anxiety nervousness feeling of anguish or disgust

2 Causes connected with *the environment*

(*a*) The child e g illness

(*b*) The husband siblings or other close relatives or friends advice or pressure to wean

(*c*) The Child Health Centre (CHC) e g advice to wean because of poor weight gain

3 Causes connected with *the interplay between the mother and her environment*

Assigned to this group were cases where the course of breast feeding was influenced by environmental factors but where at the same time the mother was easily disturbed owing to various factors Some such mothers for instance had poor motivation for or poor knowledge about breast feeding had little or no domestic help and were living under stress for different reasons

RESULTS

A *Duration of breast feeding*

The length of the breast feeding period in the two groups is given in Table 1 It is seen that

---

[1] The questionnaire used can be obtained from the authors

Table 1 *Duration of breast feeding*

( ) On discharge from the maternity ward

	Interview group		Control group	
Time weeks	Completely breast fed (%)	Completely and partially breast fed (%)	Completely breast fed (%)	Completely and partially breast fed (%)
( )	95	97	94	99
	89	95	93	96
4	85	9?	79	87
8	77	83	69	75
1	63	79	52	66
16	51	68	39	52
0	36	60	28	45
24	17	47	15	38

Partially means that cow s milk formulas were given together with breast milk

this duration was somewhat longer in the interview group and further that extremely few babies left the maternity ward solely on a diet of breast milk substitutes

B *Transient lactation crises in the interview group*

During the course of lactation 46 of the 75 mothers ran into breast feeding difficulties on a total of 69 occasions (Table 2) Seventeen mothers had more than one episode of this kind The crises could be recorded and analysed through the weekly contacts When the mother herself contacted the interviewer she was usually in immediate need of help and advice In most cases there were obvious and often trivial causes resulting in a decreased milk output For example the mother was perhaps temporarily tired or the child was crying for unusually long periods for no obvious reason Sometimes however more worrying problems either alone or as part of a chain of events caused a temporary reduction of the milk output (Table 2) It appeared and this is of fundamental importance that each individual mother reacted in her own special way to motherhood and to difficult situations Certain mothers enjoyed their maternal role and were able to quietly endure the child s crying night feeds sore nipples etc while other mothers were tied to and constantly bothered about weight curves grams time schedules the child s crying and frequent breast feeds

The number and percentage of different reasons for transient lactation crises in the interview group are presented in Table 3 Emotional disturbances in the mother (39%) and problems concerning the child (32%) were predominant Most of the crises occurred during the first months—52% before two months and 77% before three By general support and simple advice it was often possible to postpone weaning for a considerable time In 53 instances out of 69 final cessation of breast feeding was delayed by 4 weeks or more and in 25 cases by 12 weeks or more

C *Reasons for curtailed breast feeding*

Table 3 shows how the reasons for curtailment of breast feeding in the interview and control groups were distributed Reasons emanating from the interplay between the mother and the environment and rational reasons were the most common in both groups It is striking that emotional reasons were more common in the interview group than in the retrospectively interviewed control group

A brief review of the reasons or events

Table 2 *Reasons for lactation crises*

	Time of crisis (weeks from birth)	Duration (weeks) of Complete breast feeding	Partial breast feeding
REASONS CONNECTED WITH THE MOTHER			
Medical (n=10)			
Gastroenteritis vomiting Doubtful about continuing breast feeding	9	>24	–
Breasts tender Anxious	9	23	2
Breasts tender	18	20	4
Gastroenteritis Worried in case milk disappears and should she breast feed?	11	20	3
Breasts tender & inflamed Anxious—can she breast feed?	3	18	2
Breasts sore Anxious—will it affect the milk supply?	3	16	4
Rheumatoid arthritis Usually takes analgesics dares not during breast feeding Thinks milk has diminished	5	14	10
Breasts tender and sore Thinks milk has lessened Tries breast pump	4	14	10
Fever 39 5 C Should she breast feed?	7	7	7
Breasts tender	5	6	1
Emotional (n=27)			
Irritated & anxious about breast feeding Insufficient milk in the evening?	5	>24	–
Anxiety & apathy Often dislikes breast feeding Unable to sleep	20	20	4
Worried about coming holiday and helping parents Milk has lessened in amount	19	20	4
Worries about how much milk the baby is getting Baby vomits after each feed	4	20	4
Depressed Sister has had a miscarriage Feels no contact with her older child Now has less milk	13	18	6
Anxiety & apathy Upset that she cannot keep up with the rest of the family Does not want to stop breast feeding entirely	18	18	2
Tired of breast feeding doesn t know why Thinks it would be nice to finish	15	18	2
Worried about having to breast feed so often Is there anything wrong with the milk?	9	16	4
Anxious about the breast feeding and whether the baby will go up in weight—*baby refusing to suck Breast pump used several times* a day but the milk has not increased	11	14	10
Nervous & irritated about breast feeding and not knowing whether the baby has had enough	10	14	10
Tired of breast feeding feels very tired	10	12	1
Nervous as she thinks she may be pregnant again	8	12	1
Has stopped using the breast pump between feeds—finds it unpleasant The milk has suddenly dried up completely Very anxious	4	12	1
Very nervous disturbed by the child s dependence The child is restless wakes at night and cries often	16	11	13
Worried about the amount of milk The child has started wanting a night feed again	11	11	13
Worried about amount of milk Obsessed by weighing before and after feeds	10	11	2
Anxious The child has not gained sufficient weight	6	11	1
Tired of breast feeding wants to stop	14	10	5
Anxious about time spent on breast feeding while older child is demanding attention Would like to give the bottle occasionally	3	10	5
Depressed Tired of frequent breast feeding during the night Wants to give a bottle in the evening so that the child will sleep longer	9	9	8
Tired of breast feeding Baby fretful after every feed Has started to weigh the baby again before and after feeds	10	9	5
Finds breast feeding mentally tiring—would almost like to stop	6	9	5
Very worried about the amount of milk—the child whines despite 7 breast feeds per day The mother is anxiously checking the feeds by weighing	4	9	5

Table 2 *Cont*

	Time of crisis (weeks from birth)	Duration (weeks) of	
		Complete breast feeding	Partial breast feeding
Anxious She does not have as much milk as she should have a cording to the table	6	9	3
Tired of breast feeding Would like to give supplementary feeds	5	6	3
Dislikes breast feeding Feels as if her breasts are going to crack"	3	5	18
Desperate The child has lost weight CHC nurse has advised breast feeding e ery ? h—this is too much for the mother	[illegible]	2	2
REASONS CONNECTED WITH THE ENVIRONMENT			
Child (n=??)			
Child has a cold and is refusing to suck Mother worried about the child s feeds during a planned visit to friends with the family	18	>24	–
Child needs feeding every ? h Mother exhausted	14	>24	–
Child has a fever and cough and not sucking well The mother is afraid her milk will dry up	1	>24	–
Child has not gained sufficient weight	7	> 4	–
Child is discontented in the evening Will not suck more than 5 times a day Mother tired would like to start a bottle	5	>24	–
Child discontented Does not seem satisfied Mother worried	5	>24	–
Child spits out the nipple Mother tired and not sleeping	5	>24	–
Child cries is not satisfied Awake at night sleeps in the daytime	4	>24	–
Child has begun to cry ? h after each feed Mother tired milk d minishing	5	>24	–
Child in hospital with fungal infection	7	2[illegible]	[illegible]
Child has refused to feed all day Mother desperate	16	18	2
Child discontented cries Mother depressed believes she has no milk	11	18	2
Child refusing to suck Mother irritated convinced she has less milk	16	16	1
Child wanting night feeds again cries a lot Mother depressed thinks her milk has diminished	8	16	1
Child cries all the afternoons despite breast feeding every 3 h	[illegible]	1?	9
Child refused to suck at the maternity ward	[illegible]	11	13
Child crying not satisfied Mother would like to give a bottle occasionally Baby s sister jealous	11	11	3
Child refuses to such Mother has started menstruating and has less milk Wants to start weaning	10	11	2
Child still wanting night feeds Mother tired of breast feeding	11	10	5
Child has not gained sufficient weight CHC nurse has prescribed supplementary feeds	5	6	2
Child crying often Mother tired thinks that she has insufficient milk	6	5	18
Ch ld cries after every feed Mother anxious would like to start with the bottle	[illegible]	2	4
Hu band siblings etc (n=5)			
The mother s mother visiting—negative to breast feeding and disturbing influence Mother tired	4	17	7
Had to do some gardening Exhausted milk dried up a little	3	11	1
Fallen out with her mother in law Milk dried up	6	7	8
Tired and disturbed by older children Never has a chance to rest The baby is fretful in the evenings	4	5	11
Home help has left and the mother now has 5 children to look after Child fretful after meals	3	2	3
REASONS CONNECTED WITH INTERPLAY BETWEEN MOTHER AND ENVIRONMENT (n=5)			
Stressed and nervous after christening with many guests Milk d minished	9	?0	4
The mother is on holiday The baby is off balance and the mother anxious	1[illegible]	16	4
Mother has started menstruating Hot summer Mother in law on the way Stressed M lk drying up	13	13	11
On h l day with family Tired and stressed Child fretful	?3	1?	6
Chr tmas stress Child wh ning Tired of breast feed ng "every 15 m nutes"	8	6	3

Table 3 *Reasons for transient lactation crises and curtailed breast feeding (before 6 months)*

	Lactation crises Interview group		Curtailed breast feeding Interview group		Curtailed breast feeding Control group	
Reason connected with	*n*	%	*n*	%	*n*	%
The mother						
Medical	10	14	3	7	4	7
Rational	0	0	12	26	16	27
Emotional	27	39	10	22	5	8
The environment						
Child	22	32	0	0	0	0
Husband siblings etc	5	7	0	0	2	3
Child Health Centre	0	0	1	2	2	3
The interplay between mother and environment	5	7	20	43	31	52
	69	100	46	100	60	100

that finally resulted in a complete cessation of breast feeding in each individual mother is given in Table 4

COMMENTS

From the findings in a previous study we concluded that the true or immediate reasons for curtailed breast feeding were difficult to reveal in retrospect (1) By the retrospective method it was only possible to define in more general sociological terms the kind of mothers who tended to breast feed their infants for longer or shorter periods of time The present investigation has clearly demonstrated that by the prospective approach with continuous and intense contact *with every mother it is* as a rule easy to identify the cause or causes leading to a lactation crisis or weaning

An analysis of the brief case histories presented in Tables 2 and 4 shows above all a great variety of immediate reasons for early weaning Sometimes several problems in combination seemed to form the causative factor and in some cases there was a chain of more or less harmless events that sooner or later resulted in a lactation crisis or weaning Problems connected with the child obviously played a greater role as the cause of transient lactation crises than when the crises ended in permanent termination of breast feeding (Table 3) It also seemed that emotional reasons were much more common as a cause of lactation crises in the interview group than in the control group in which the mothers were interviewed for the first time 6 months after the birth of the child The mothers themselves seem to have difficulties in recognising or accepting in retrospect the emotional strains that may be involved in breast feeding

A detailed study of the case histories revealed that many mothers had a tendency to exaggerate the importance of minor illnesses in themselves or in the child Anxiety was often due to rather trivial or seemingly irrelevant incidents If for example the child *vomited a little after some meals* if the child refused to suck at a few meals or started to cry for some unknown reason then some mothers tended to focus all their attention on the feeding and these unnecessary worries often ended up in a lactation crisis In some cases even minor deviations from the ordinary habits of life caused an interruption of breast feeding Visits or undue intervention by relatives and the demands placed on the mother during holidays or other celebrations seemed to be particularly harmful to breast feeding Also the needs of other children in the family prompted some mothers to wean early In

Table 4 *Reasons for termination of breast feeding before 6 months in the interview group*

	Duration (weeks) of	
	Complete breast feeding	Partial breast feeding thereafter
REASONS CONNECTED WITH THE MOTHER		
Medical (n=3)		
Retracted nipples	0	1
Neurosis	0	0
Post partum psychosis	0	0
Average	0.0	0.3
Rational (n=12)		
Studies	20	4
Employment	20	4
Considers she has breast fed long enough Wants to take driving lessons	20	3
Considers she has breast fed long enough Wants to attend union meetings	18	6
Employment and studies	16	4
Breast feeding terminated because of the child s age	15	6
Studies	13	7
Studies	12	7
Employment	10	4
Studies	5	18
Considers she has breast fed long enough Large family to look after	5	11
Mother of 5 with much domestic work and no home help	2	3
Average	12.8	6.5
Emotional (n=10)		
Feelings of restlessness and stress—not enough time to do what she likes Can t stand breast feeding any longer	18	2
Does not get on well with breast feeding gets tired of constraint and irregular hours Wants to be free for the summer	16	4
Cannot stand the child s total dependence	11	13
Depressed and uncomfortable with breast feeding Older child jealous	11	3
Breast feeding feels like a burden hard work one s physical strength disappears Feels disturbed by the child s total dependence	9	9
Previous depression afraid of having a relapse The child sucks the juice out of me	9	5
Finds breast feeding unpleasant slushy Wants to be free for the vacation	9	2
Hard and tiresome to breast feed Can t manage to breast feed frequently the child is underweight and is prescribed bottle feeds	6	2
Feelings of disgust for breast feeding	2	1
Dislikes breast feeding from before This child jaundiced after birth and the mother stops breast feeding immediately	1	0
Average	9.2	4.1
REASONS CONNECTED WITH THE ENVIRONMENT		
Child health centre (n=1)		
Wanted to breast feed but CHC persistently urged her to give supplementary feeds as the child was underweight	12	9
REASONS CONNECTED WITH THE INTERPLAY BETWEEN MOTHER AND ENVIRONMENT (n=19)		
The mother felt constrained all the time Told by the CHC to wean at 4 months but did not wean until 5 months having misunderstood an information leaflet	20	4
Repeated refusal to suck The mother gets depressed and long to be back home in the country CHC encourages her to give the bottle advice followed at once	18	2
The mother did not want to breast feed more than 3 meals and started weaning The child refused to suck and the mother dried up	17	4

Table 3 *Reasons for transient lactation crises and curtailed breast feeding (before 6 months)*

	Lactation crises Interview group		Curtailed breast feeding Interview group		Curtailed breast feeding Control group	
Reason connected with	*n*	%	*n*	%	*n*	%
The mother						
Medical	10	14	3	7	4	7
Rational	0	0	12	26	16	27
Emotional	27	39	10	22	5	8
The environment						
Child	22	32	0	0	0	0
Husband siblings etc	5	7	0	0	2	3
Child Health Centre	0	0	1	2	2	3
The interplay between mother and environment	5	7	20	43	31	52
	69	100	46	100	60	100

that finally resulted in a complete cessation of breast feeding in each individual mother is given in Table 4

COMMENTS

From the findings in a previous study we concluded that the true or immediate reasons for curtailed breast feeding were difficult to reveal in retrospect (1) By the retrospective *method it was only possible to define in more* general sociological terms the kind of mothers who tended to breast feed their infants for longer or shorter periods of time The present investigation has clearly demonstrated that *by the prospective approach* with continuous and intense contact with every mother it is as a rule easy to identify the cause or causes leading to a lactation crisis or weaning

An analysis of the brief case histories presented *in* Tables 2 and 4 shows above all a great variety of immediate reasons for early weaning Sometimes several problems in combination seemed to form the causative factor and in some cases there was a chain of more or *less harmless* events that sooner or later resulted in a lactation crisis or weaning Problems connected with the child obviously played a greater role as the cause of transient lactation crises than when the crises ended *in permanent termination* of breast feeding (Table 3) It also seemed that emotional reasons were much more common as a cause of lactation crises in the interview group than in the control group in which the mothers *were interviewed for the first* time 6 months after the birth of the child The mothers themselves seem to have difficulties in recognising or accepting in retrospect the emotional strains that may be involved in breast feeding

A detailed study of the case histories revealed that many mothers had a tendency to exaggerate the importance of minor illnesses in themselves or in the child Anxiety was often due to rather trivial or seemingly irrelevant incidents If for example the child vomited a little after some meals if the child refused to suck at a few meals or started to cry for some unknown reason then some mothers tended to focus all their attention on the feeding and these unnecessary worries often ended up in a lactation crisis In some cases even minor deviations from the ordinary habits of life caused an interruption of breast feeding Visits or undue intervention by relatives and the demands placed on the mother during holidays or other celebrations seemed to be particularly harmful to breast feeding *Also* the needs of other children *in the family* prompted some mothers to wean early In

mothers also seemed to need help in their efforts to reach a sensible decision

The most impressive experience gained during this investigation was that each individual mother seemed to react in her own individual way to problems Some were extremely sensitive and needed much support while others seemed able to cope with fairly severe strain without much ado The differences might have been a question of disposition or constitution To what extent a sense of security and knowledge about breast feeding played a role cannot be decided from the study

In 1972 only four per cent of the mothers in Uppsala still nursed their babies at 24 weeks while in the present series from 1974 the corresponding figure for the control group was 38% and for the interview group 47% The difference between the control and interview group is probably due to the more regular and intense contacts maintained by the interviewer in the latter group The marked increase in the average duration of breast feeding over a period of a few years is certainly primarily and basically the result of an altered attitude among young women towards breast feeding This change began slowly partly thanks to the activities of La Leche League but was speeded up quickly by the help of mass media and also by measures taken by national and local health authorities It is interesting to note that the baby food manufacturers have not attempted any counter activities although the sale of breast milk substitutes during the last few years had decreased markedly On the contrary they have accepted the development with understanding willingness However this study has shown that even if mothers in general really want to breast feed their infants this fact is no guarantee for success Many mothers obviously need much direct support and advice to be able to complete a reasonably long lactation period

In brief our conclusion is that breast feeding is most likely to succeed and to be continued if the mothers have learned and accepted the concept that breast feeding is superior to artificial feeding during the first months of life and if they are offered immediate and competent practical help in the event of feeding problems or other difficulties

REFERENCE

1 Sjolin S Hofvander Y & Hillervik C Factors related to early termination of breast feeding A retrospective study in Sweden *Acta Paediatr Scand* 66 505 1977

Submitted Nov 16 1978
Accepted Febr 1 1979

(S S) Department of Paediatrics
University Hospital
S-750 14 Uppsala
Sweden

Table 4 *Cont*

	Duration (weeks) of	
	Complete breast feeding	Partial breast feeding thereafter
The child refused to suck for long period over Christmas Pressure from grandparents and husband to give breast milk replacer When home again the mother tries to breast feed wholly but the child refuses and continues to prefer the bottle and the mother gives up	16	1
The mother finds breast feeding tiresome when there is not enough milk Does not want to feed frequently	12	6
The mother young nervous and stressed about poor relations with her husband The child whining the mother feeds frequently without result	12	1
The child starts to wake up at night The mother is menstruating and has less milk Feels tied to the question of milk amount	11	2
The child cries day and night The mother anxious breast feeds frequently as the child was found to be underweight at CHC Advice from CHC to weigh breast feeds and to give the bottle or stop breast feeding The mother tired of the frequent breast feeding and of the other children and stops breast feeding	11	1
The child still wants night feeds at 3 months Older siblings demanding attention	10	5
The mother tired has little help child crying at night long period of mixed feeding The child gets pneumonia and is admitted to hospital The mother doesn t want to go there for breast feeding	9	8
Whining child The mother has breast fed every 3 h for 2 days Husband dislikes both the breast feeding and the survey The mother despaired and introduces the bottle	7	8
The mother unwell CHC finds the child underweight The mother has tried frequent breast feeding without result Convinced she is not able to breast feed	7	7
The mother irritated about breast feeding has a cold feels tired too much Christmas cleaning Breast fed frequently for 6 days without result The child quieter after start of bottle feeding	6	3
The mother sensitive and nervous before Christmas and the gathering of many relatives Dries up and the child is whining Pressure from various people to give the bottle	4	1
The child not content despite 6–9 feeds a day 1 hour each time The mother tired and dries up suddenly	3	2
The child refuses to suck temporarily the mother gives up and does not want to try again (feels averse to the child)	3	0
Whining child mother anxious about the milk amount wants to participate in the local spring festival and starts the child on the bottle	2	4
The mother wants to breast feed but cannot relax is nervous tense and anxious about the milk amount Siblings jealous when the baby is fed The child cries 4 persons living in the same room The mother dries up	2	2
Whining child demanding siblings no domestic help no time to rest	0	6
Average	9 0	3 6

many instances the mother did not feel at ease with breast feeding Some of them felt too tied while others were unable to stand the child s total dependence Some mothers found breast feeding unpleasant In a few cases an insufficiently understanding husband was obviously a major reason for early weaning

In many cases weaning could be avoided by simple advice and general support This was true especially when the mother was unduely worried or when the immediate problem could be easily eliminated

Sometimes all attempts to help failed completely This happened in particular with mothers who for some reason did not really want to go on with breast feeding In other instances early weaning seemed to be unavoidable for quite rational reasons or owing to a pressing situation in the family These

Acta Pædiatr Scand 68 531–539 1979

SOCIAL BACKGROUND AND LIFE EVENTS OF CHILDREN ADMITTED TO A PAEDIATRIC DEPARTMENT

J AAGAARD

From the Department of Paediatrics Randers Centralsygehus Randers and the Institute of Social Medicine University of Aarhus Aarhus Denmark

ABSTRACT Aagaard J (Department of Paediatrics Randers Centralsygehus Denmark and Institute of Social Medicine University of Aarhus Denmark) Social background and life events of children admitted to a paediatric department Acta Paediatr Scand 68 531 1979 —During a period of one year a questionnaire was filled in by parents of children who were more than one year old and admitted to the paediatric department in Randers Denmark The questionnaire was especially concerned with factors describing the social background of the family and with selected life events Family background variables such as low level of parents education and low income were found to be associated with psychosomatic and psychic diseases of the children No associations between these negative family background variables and respiratory tract infections were found The data show a strikingly high frequency of selected life events Noticeable intercorrelations were found between social factors life events and diagnostic groups In the light of this it was not considered meaningful to analyze life events independently of the family background variables A combined measurement for family background variables and selected life events was strongly associated with psychosomatic and psychic diseases The additional information due to life events over the information due to family background variables was pronounced It was remarkable that stressful life events also were frequent among children with some somatic diseases The inference of these results is a plea for application of analysis of individual social history to more hospitalized children The results might further have implications for preventive work with respect to the changes which seem to have taken place in important social conditions

KEY WORDS Social paediatrics life events hospital admission

Many investigations have dealt with social background factors for children admitted to hospital (11 14) The reported investigations are however criticizable from a methodological point of view One usually notes especially that only more stable social variables are taken into account and there is no attempt to evaluate the relative significance of the elements in the social history of the child

It has been shown in some investigations that many children in a paediatric department have prior psychic deviating development and/or emotional disturbances (9 13) Thus Apley & McKeith (1) have emphasized that various organic symptoms often have a psychic background

In addition to this aspect there seems in some cases to be a connection between earlier diseases deviating development and later symptoms in pedagogic social or health areas (3) It must however be stressed that information dealing with prediction of later illness or impaired development from essential elements in the social history is only available for certain diagnoses (10 12)

Purpose and definitions

In this part of the investigation the purpose was to estimate the relative significance of social background factors and life events for certain diagnostic groups of children admitted to the department

By *social factors* is meant relatively stable aspects of the parents situation such as level of education income social status and residence

Table 1 *Diagnostic group by the parents' education*

Parents' education	Respiratory tract infections	Infections outside respiratory tracts	Psychosomatic and psychic diseases	Other diseases	Total number per cent	χ^2 test (corrected)
Father's school education						
≤9 years	21	32	44	135	232 (65.4)	χ^2 21.0
>9 years	19	10	2	54	85 (23.9)	$df=3$
Not stated/not relevant	7	5	9	17	38 (10.7)	$p<0.001$
Total	47 (13.[illegible])	47 (13.2)	55 (15.5)	206 (58.0)	355 (100.0)	
Father's occupational education						
≤1 year	6	10	22	65	103 (29.0)	$\chi^2=12.7$
>1 year	35	3[illegible]	24	124	215 (60.6)	$df=3$
Not stated/not relevant	6	5	9	17	37 (10.4)	$p<0.01$
Total	47 (13.2)	47 (13.2)	55 (15.5)	206 (58.0)	355 (100.0)	
Mother's school education						
≤9 years	27	27	51	153	258 (72.7)	$\chi^2=21.7$
>9 years	20	19	4	52	95 (26.8)	$df=3$
Not stated	0	1	0	1	2 (0.6)	$p<0.001$
Total	47 (13.2)	47 (13.2)	55 (15.5)	206 (58.0)	355 (100.0)	
Mother's occupational education						
≤1 year	[illegible]0	4	39	1[illegible]1	204 (57.5)	$\chi^2=10.1$
>1 year	6	22	14	8	144 (40.6)	$df=3$
Not stated	1	1	[illegible]	3	7 (1.9)	$p<0.05$
Total	47 (13.2)	47 (13.2)	55 (15.5)	206 (58.0)	355 (100.0)	

As shown in Table 1, highly significant differences were found between diagnostic groups with respect to length of the parents' school education, and significant differences with respect to the length of the parents' occupational education.

The length of school education was uniform for men and women, whereas a greater part of men than women had a longer occupational education. This corresponds to the condition of the area population.

In the four diagnostic groups psychosomatic and psychic diseases were marked by an excess of parents with short education and training, while infections of the respiratory tract contained predominance of parents with a higher education.

Table 2 shows diagnostic groups by selected family background variables. The dividing value for taxable income at 60000 D kr. corresponds to the average taxable income for an unskilled worker. As shown in Table 2, income is the only variable which distinguishes significantly between diagnostic groups. This association was characterized in the fact that children with psychosomatic and psychic diseases more frequently had parents with a lower income.

Place of residence was judged partly by number of rooms and partly by overcrowding, defined as more than one person per room. Neither parameter distinguished significantly between diagnostic groups.

Frequencies of life events

An estimate of the frequency of life events among Danish children is not yet available.

Among preschool children the percentage frequencies of hospitalization of brother or sister were 10.1% and of parents 22.8%

By *life events* is meant social events such as divorce unemployment and changes in inter personal relationships

METHOD

The investigation consisted of questionnaires filled in by parents of children admitted to the paediatric department or admitted to the surgical department for herniotomy The hospital receives children from a geographically re latively well defined area There is no paediatric out patients clinic in the area There is no social selection for admission to hospital

Prior to this investigation a pilot study was undertaken and published (15 16) The questionnaire dealt with rela tively stable social background variables and changes in the circumstances of life which had occurred during the year prior to admission A slightly modified edition of Coddington s life event list was used (4 5) A critical evaluation of Coddington s life event/life change unit (LCU) method has been published earlier (17)

The questionnaires were distributed by the head nurse of the department The procedure followed was in ac cordance with the demands of the Helsinki II Declaration (8)

For the non response group selected social variables were obtained from the medical record For all patients diagnosis admission type and duration of stay were re corded

The validity of each diagnosis was confirmed by the chief physician of the department The main diagnoses were aggregated in diagnostic groups The group respira tory tract infections consisted of 38 preschool children (age 1–6) of whom 25 had lower and 13 had upper res piratory tract infections and 9 elementary school chil dren (age 7–14) of whom 7 had lower and 2 had upper respiratory tract infections The group of infections out side the respiratory tract consisted of 31 preschool chil dren of whom 8 had gastroenteritis and 11 had urinary tract infections and 16 elementary school children of whom 5 had infections of the urinary tract and 5 had meningitis/sepsis The group psychosomatic and psychic diseases consisted of 20 preschool children of whom 7 had enuresis and 6 encopresis and 35 elementary school chil dren of whom 13 had abdominal colic 12 had enuresis and 6 had infantile neurosis The group other diseases consisted of 139 preschool children of whom 5 had a diagnosis of accidental drug poisoning 12 had other forms of poisoning 12 had various symptoms from the gastro intestinal tract 30 had febrile convulsions and 9 had epilepsy and 67 elementary school children of whom 9 had epilepsy 10 had asthma bronchiale 5 were admitted for herniotomy and 5 had diabetes mellitus

The reliability of the information given in the ques tionnaire was judged by comparing this information with the analogous data from the medical record Social back ground variables showed a great deal of agreement be tween the record data and questionnaire data The re liability of the information concerning life events could not be estimated as the majority of records was incom plete on this point

Statistical procedures

The material was processed at the Regional EDP Center University of Aarhus (RECAU) The program for ap propriate scoring was written by Professor Johs Ipsen A further description of the statistical procedures is pub lished elsewhere (17)

By a *profile* of a diagnostic group is meant the array of normalized mean for the independent variables The variables used in this investigation were all dichotomized The normalized means are random variates of a normal distribution with mean equal to zero and unit standard error

Combined variables were computed These variables were the sum of selected profile elements multiplied by the appropriate coefficient for the individual element The appropriate coefficients were calculated by a process which corrected for intercorrelations between the inde pendent variables The combined measurements might in this connection be understood as a weighted index which takes negative social factors and selected life events into account

MATERIAL

The patient group consisted of all children over one y of age who were admitted in the period Oct 1st 19 Sept 30th 1977

Excluded from participation in the investigation w 1) Children of foreign language speaking parents 2) stitutionalized children 3) Children transferred to other hospital within the first day after admission Children who were readmitted during the period of inv tigation 5) Children who stayed in hospital less than hours 6) Children who had participated in the pilot stu (16) In the period of investigation 482 children were mitted and 22 (4 6%) were excluded

The occupational group distribution of the fathers of children corresponded to that of men in the same group in the population of the area (6)

Five (1 1%) of the children s parents did not want participate in the investigation 455 questionnaires w distributed 375 (82 4%) of these were returned 14 (3 7 of the returned questionnaires were incompletely fill in Family background variables did not differ significa ly between response and non response groups

Of the response group s 361 patients 187 were bo and 174 were girls The average age was 5 3 years (S D 3 8) The average stay in hospital was 6 9 days (S D 4 6) 228 (63 2%) of the children were at preschool ag 127 (35 2%) were at elementary school age and 6 (1 6 were older As the significance of life events among oth things is age dependent (5) the children over 14 years age were excluded from the following analysis The r duced study population then consisted of 355 patients

RESULTS

Diagnostic groups by social factors

The children s main diagnoses at time of dis charge from hospital are in Tables 1 and 2 re duced to three groups and a remainder group

Table 1 *Diagnostic group by the parents education*

Parents education	Respiratory tract infections	Infections outside respiratory tracts	Psychosomatic and psychic diseases	Other diseases	Total number per cent	χ^2 test (corrected)
Father s school education						
≤9 years	21	32	44	135	232 (65 4)	χ^2=21 0
>9 years	19	10	2	54	85 (23 9)	df=3
Not stated/not relevant	7	5	9	17	38 (10 7)	p<0 001
Total	47 (13 2)	47 (13 2)	55 (15 5)	206 (58 0)	355 (100 0)	
Father s occupational education						
≤1 year	6	10	22	65	103 (29 0)	χ^2=12 7
>1 year	35	32	4	124	215 (60 6)	df=3
Not stated/not relevant	6	5	9	17	37 (10 4)	p<0 01
Total	47 (13 2)	47 (13)	55 (15 5)	06 (58 0)	355 (100 0)	
Mothe s school education						
≤9 years	27	27	51	153	258 (72 7)	χ^2=21 7
>9 years	20	19	4	52	95 (26 8)	df=3
Not stated	0	1	0	1	2 (0 6)	p<0 001
Total	47 (13 2)	47 (13 2)	55 (15 5)	206 (58 0)	355 (100 0)	
Mother s occupational education						
≤1 year	20	24	39	121	204 (57 5)	χ^2=10 1
>1 year	26	2	14	82	144 (40 6)	df=3
Not stated	1	1	2	3	7 (1 9)	p<0 05
Total	47 (13 2)	47 (13 2)	55 (15 5)	206 (58 0)	355 (100 0)	

As shown in Table 1 highly significant differences were found between diagnostic groups with respect to length of the parents school education and significant differences with respect to the length of the parents occupational education

The length of school education was uniform for men and women whereas a greater part of men than women had a longer occupational education This corresponds to the condition of the area population

In the four diagnostic groups psychosomatic and psychic diseases were marked by an excess of parents with short education and training while infections of the respiratory tract contained predominance of parents with a higher education

Table 2 shows diagnostic groups by selected family background variables The dividing value for taxable income at 60000 D kr corresponds to the average taxable income for an unskilled worker As shown in Table 2 income is the only variable which distinguishes significantly between diagnostic groups This association was characterized in the fact that children with psychosomatic and psychic diseases more frequently had parents with a lower income

Place of residence was judged partly by number of rooms and partly by overcrowding defined as more than one person per room Neither parameter distinguished significantly between diagnostic groups

Frequencies of life events

An estimate of the frequency of life events among Danish children is not yet available

Among preschool children the percentage frequencies of hospitalization of brother or sister were 10 1% and of parents 22 8%

By *life events* is meant social events such as divorce unemployment and changes in inter personal relationships

METHOD

The investigation consisted of questionnaires filled in by parents of children admitted to the paediatric department *or admitted to the surgical department for herniotomy* The hospital receives children from a geographically re latively well defined area There is no paediatric out patients clinic in the area There is no social selection for admission to hospital

Prior to this investigation a pilot study was undertaken and published (15 16) The questionnaire dealt with rela tively stable social background variables and changes in the circumstances of life which had occurred during the year prior to admission A slightly modified edition of Coddington s life event list was used (4 5) A critical evaluation of Coddington s life event/life change unit (LCU) method has been published earlier (17)

The questionnaires were distributed by the head nurse of the department The procedure followed was in ac cordance with the demands of the Helsinki II Declaration (8)

For the non response group selected social variables were obtained from the medical record For all patients diagnosis admission type and duration of stay were re corded

The validity of each diagnosis was confirmed by the chief physician of the department The main diagnoses were aggregated in diagnostic groups The group respira *tory tract infections consisted of 38 preschool children* (age 1–6) of whom 25 had lower and 13 had upper res piratory tract infections and 9 elementary school chil *dren* (age 7–14) of whom 7 had lower and 2 had *upper* respiratory tract infections The group of infections out side the respiratory tract consisted of 31 preschool chil dren of whom 8 had gastroenteritis and 11 had urinary tract infections and 16 elementary school children of whom 5 had infections of the urinary tract and 5 had meningitis/sepsis The group psychosomatic and psychic diseases consisted of 20 preschool children of whom 7 had enuresis and 6 encopresis and 35 elementary school chil dren of whom 13 had abdominal colic 12 had enuresis and 6 had infantile neurosis The group other diseases consisted of 139 preschool children of whom 5 had a diagnosis of accidental drug poisoning 12 had other forms of poisoning 12 had various symptoms *from the gastro* intestinal tract 30 had febrile convulsions and 9 had epilepsy and 67 elementary school children of whom 9 had epilepsy 10 had asthma *bronchiale 5 were admitted* for herniotomy and 5 had diabetes mellitus

The reliability of the information given in the ques tionnaire was judged *by comparing* this information with the analogous data from the medical record Social back ground variables showed a great deal of agreement be tween the record data and questionnaire data The re liability of the information concerning life events could not be estimated as the majority of records was incom *plete on this point*

Statistical procedures

The material was processed at the Regional EDP-Center University of Aarhus (RECAU) The program for ap propriate scoring was written by Professor Johs Ipsen A further description of the statistical procedures is pub lished elsewhere (17)

By a profile of a diagnostic group is meant the array of normalized mean for the independent variables The variables used in this investigation were all dichotomized The normalized means are random variates of a normal distribution with mean equal to zero and unit standard error

Combined variables were computed These variables were the sum of selected profile elements multiplied by the appropriate coefficient for the individual element The appropriate coefficients were calculated by a process which corrected for intercorrelations between the inde pendent variables The combined measurements might in this connection be understood as a weighted index which takes negative social factors and selected life events into account

MATERIAL

The patient group consisted of all children over one year of age who were admitted in the period Oct 1st 19 6 Sept 30th 1977

Excluded from participation in the investigation were 1) Children of foreign language speaking parents 2) In stitutionalized children 3) Children transferred to an *other hospital within the first* day after admission 4) Children who were readmitted during the period of inves tigation 5) Children who stayed in hospital less than 24 hours 6) Children who had participated in the pilot study (16) In the period of investigation 482 children were ad mitted and 22 (4 6%) were excluded

The occupational group distribution of the fathers of the children corresponded to that of men in *the same age* group in the population of the area (6)

Five (1 1%) of the children s parents did not want to participate in the investigation 455 questionnaires were distributed 375 (82 4%) of these were returned 14 (3 7%) of the returned questionnaires were incompletely filled in Family background variables did not differ *significant* ly between response and non response groups

Of the response group s 361 patients 187 were boys *and 174 were girls The average age was* 5 3 years (S D = 3 8) The average stay in hospital was 6 9 days (S D = 4 6) 228 (63 2%) of the children were at preschool age 127 (35 2%) were at elementary school age and 6 (1 6%) were older As the significance of life events among other things is age dependent (5) the children over 14 years of age were excluded from the following analysis The re duced study population then consisted of 355 patients

RESULTS

Diagnostic groups by social factors

The children s main diagnoses at time of dis charge from hospital are in Tables 1 and 2 re duc[illegible]

Table 1 *Diagnostic group by the parents education*

Parents education	Respiratory tract infections	Infections outside respiratory tracts	Psychosomatic and psychic diseases	Other diseases	Total number per cent	χ^2 test (corrected)
Fathe s school education						
≤9 years	[illegible]1	3	44	135	23[illegible] (65 4)	χ^2=21 0
>9 years	19	10		54	85 ([illegible]3 9)	df =3
Not stated/not relevant	7	5	9	17	38 (10 7)	p<0 001
Total	47 (13 [illegible])	47 (13 [illegible])	55 (15 5)	[illegible]06 (58 0)	355 (100 0)	
Father s occupational education						
≤1 year	6	10	[illegible]2	65	103 (9 0)	χ^2=12 7
>1 year	35	3[illegible]	4	1[illegible]4	215 (60 6)	df =3
Not stated/not relevant	6	5	9	17	37 (10 4)	p<0 01
Total	47 (13 [illegible])	47 (13 [illegible])	55 (15 5)	[illegible]06 (58 0)	355 (100 0)	
Mothe s school education						
≤9 years	[illegible]7	[illegible]7	51	153	258 (7[illegible] 7)	χ^2=[illegible]1 7
>9 years	20	19	4	52	95 ([illegible]6 8)	df =3
Not stated	0	1	0	1	2 (0 6)	p<0 001
Total	47 (13 [illegible])	47 (13 [illegible])	55 (15 5)	[illegible]06 (58 0)	355 (100 0)	
Mother s occupational education						
≤1 year	[illegible]0	[illegible]4	39	1[illegible]1	[illegible]04 (57 5)	χ^2=10 1
>1 year	[illegible]6	2	14	8	144 (40 6)	df =3
Not stated	1	1	[illegible]	3	7 (1 9)	p<0 05
Total	47 (13 [illegible])	47 (13 [illegible])	55 (15 5)	[illegible]06 (58 0)	355 (100 0)	

As shown in Table 1 highly significant differences were found between diagnostic groups with respect to length of the parents school education and significant differences with respect to the length of the parents occupational education

The length of school education was uniform for men and women whereas a greater part of men than women had a longer occupational education This corresponds to the condition of the area population

In the four diagnostic groups psychosomatic and psychic diseases were marked by an excess of parents with short education and training while infections of the respiratory tract contained predominance of parents with a higher education

Table 2 shows diagnostic groups by selected family background variables The dividing value for taxable income at 60000 D kr corresponds to the average taxable income for an unskilled worker As shown in Table 2 income is the only variable which distinguishes significantly between diagnostic groups This association was characterized in the fact that children with psychosomatic and psychic diseases more frequently had parents with a lower income

Place of residence was judged partly by number of rooms and partly by overcrowding defined as more than one person per room Neither parameter distinguished significantly between diagnostic groups

Frequencies of life events

An estimate of the frequency of life events among Danish children is not yet available

Among preschool children the percentage frequencies of hospitalization of brother or sister were 10 1% and of parents 22 8%

By *life events* is meant social events such as divorce unemployment and changes in inter personal relationships

METHOD

The investigation consisted of questionnaires filled in by parents of children admitted to the paediatric department or admitted to the surgical department for herniotomy The hospital receives children from a geographically re latively well-defined area There is no paediatric out patients clinic in the area *There is no social selection for* admission to hospital

Prior to this investigation a pilot study was undertaken and published (15 16) The questionnaire dealt with rela tively stable social background variables and changes in the circumstances of life which had occurred during the year prior to admission A slightly modified edition of Coddington s life event list was used (4 5) A critical evaluation of Coddington s life event/life change unit (LCU) method has been published earlier (17)

The questionnaires were distributed by the head nurse of the department The procedure followed was in ac cordance with the demands of the Helsinki II Declaration (8)

For the non response group selected social variables were obtained from the medical record For all patients diagnosis admission type and duration of stay were re corded

The validity of each diagnosis was confirmed by the chief physician of the department The main diagnoses were aggregated in diagnostic groups The group respira tory tract infections consisted of 38 preschool children (age 1–6) of whom 25 had lower and 13 had upper res piratory tract infections and 9 elementary school chil dren (age 7–14) of whom 7 had lower and 2 had upper respiratory tract infections The group of infections out side the respiratory tract consisted of 31 preschool chil dren of whom 8 had gastroenteritis and 11 had urinary tract infections and 16 elementary school children of whom 5 had infections of the urinary tract and 5 had meningitis/sepsis The group psychosomatic and psychic diseases consisted of 20 preschool children of whom 7 had *enuresis and* 6 encopresis and 35 elementary school chil dren of whom 13 had abdominal colic 12 had enuresis and 6 had infantile neurosis The group other diseases consisted of 139 preschool children of whom 5 had a diagnosis of accidental drug poisoning 12 had other forms of poisoning 12 had various symptoms from the gastro intestinal tract 30 had febrile convulsions and 9 had epilepsy and 67 elementary *school children of whom* 9 had epilepsy 10 had asthma bronchiale 5 were admitted for herniotomy and 5 had diabetes mellitus

The reliability of the information given in the ques tionnaire was judged by comparing this information with the analogous data from the medical record Social back *ground* variables showed a great deal of agreement be tween the record data and questionnaire data The re liability of the information concerning life events could not be estimated as the majority of records was incom plete on this point

Statistical procedures

The material was processed at the Regional EDP Center University of Aarhus (RECAU) The program for ap propriate scoring was written by Professor Johs Ipsen A further description of the statistical procedures is pub lished elsewhere (17)

By a profile of a diagnostic group is meant the array of normalized mean for the independent variables The variables used in this investigation were all dichotomized The normalized means are random variates of a normal distribution with mean equal to zero and unit standard error

Combined variables were computed These variables were the sum of selected profile elements multiplied by the appropriate coefficient for the individual element The appropriate coefficients were calculated by a process which corrected for intercorrelations between the inde pendent variables The combined measurements might in this connection be understood as a weighted index which takes negative social factors and selected life events into account

MATERIAL

The patient group consisted of all children over one year *of age who were admitted in the period Oct* 1st 1976-Sept 30th 1977

Excluded from participation in the investigation were 1) Children of foreign language speaking parents 2) In stitutionalized children 3) Children transferred to an other hospital within the first day after admission 4) Children who were readmitted during the period of inves tigation 5) Children who stayed in hospital less than 24 hours 6) Children who had participated in the pilot study (16) In the period of investigation 482 children were ad mitted and 22 (4 6%) were excluded

The occupational group distribution of the fathers of the children corresponded to that of men in the same age *group in the population of the area* (6)

Five (1 1%) of the children s parents did not want to participate in the investigation 455 questionnaires were distributed 375 (82 4%) of these were returned 14 (3 7%) of the returned questionnaires were incompletely filled in Family background variables did not differ significant ly between response and non response groups

Of the response group s 361 patients 187 were boys and 174 were girls The average age was 5 3 years (S D = 3 8) The average stay in hospital was 6 9 days (S D = 4 6) 228 (63 2%) of the children were at preschool age 127 (35 2%) were at elementary school age and 6 (1 6%) were older As the significance of life events among other things is age dependent (5) the children over 14 years of age were excluded from the following analysis The re duced study population then consisted of 355 patients

RESULTS

Diagnostic groups by social factors

The children s main diagnoses at time of dis charge from hospital are in Tables 1 and 2 re duced to three groups and a remainder group

Table 1 *Diagnostic group by the parents education*

Parents education	Respiratory tract infections	Infections outside respiratory tracts	Psychosomatic and psychic diseases	Other diseases	Total number per cent	χ^2 test (corrected)
Father s school education						
≤9 years	21	32	44	135	232 (65 4)	χ^2=21 0
>9 years	19	10	2	54	85 (23 9)	$d f$ =3
Not stated/not relevant	7	5	9	17	38 (10 7)	p<0 001
Total	47 (13 2)	47 (13)	55 (15 5)	206 (58 0)	355 (100 0)	
Father s occupational education						
≤1 year	6	10	22	65	103 (9 0)	χ^2=12 7
>1 year	35	32	24	124	215 (60 6)	$d f$ =3
Not stated/not relevant	6	5	9	17	37 (10 4)	p<0 01
Total	47 (13 2)	47 (13 2)	55 (15 5)	206 (58 0)	355 (100 0)	
Mother s school education						
≤9 years	27	27	51	153	258 (7 7)	χ=21 7
>9 years	0	19	4	52	95 (6 8)	$d f$ =3
Not stated	0	1	0	1	2 (0 6)	p<0 001
Total	47 (13 2)	47 (13 2)	55 (15 5)	206 (58 0)	355 (100 0)	
Mother s occupational education						
≤1 year	20	4	39	171	204 (57 5)	χ^2=10 1
>1 year	6	22	14	82	144 (40 6)	$d f$ =3
Not stated	1	1	2	3	7 (1 9)	p<0 05
Total	47 (13 2)	47 (13)	55 (15 5)	206 (58 0)	355 (100 0)	

As shown in Table 1 highly significant differences were found between diagnostic groups with respect to length of the parents school education and significant differences with respect to the length of the parents occupational education

The length of school education was uniform for men and women whereas a greater part of men than women had a longer occupational education This corresponds to the condition of the area population

In the four diagnostic groups psychosomatic and psychic diseases were marked by an excess of parents with short education and training while infections of the respiratory tract contained predominance of parents with a higher education

Table 2 shows diagnostic groups by selected family background variables The dividing value for taxable income at 60000 D kr corresponds to the average taxable income for an unskilled worker As shown in Table 2 income is the only variable which distinguishes significantly between diagnostic groups This association was characterized in the fact that children with psychosomatic and psychic diseases more frequently had parents with a lower income

Place of residence was judged partly by number of rooms and partly by overcrowding defined as more than one person per room Neither parameter distinguished significantly between diagnostic groups

Frequencies of life events

An estimate of the frequency of life events among Danish children is not yet available

Among preschool children the percentage frequencies of hospitalization of brother or sister were 10 1% and of parents 22 8%

Table 2 *Diagnostic group by the parents marital status income and number of rooms in residence*

Parents marital status income and residence	Respiratory tract infections	Infections outside respiratory tracts	Psychosomatic and psychic diseases	Other diseases	Total number per cent	χ^2 test (corrected)
Marital status						
Not married	8	4	9	16	37 (10 4)	$\chi^2=5\ 9$
Married	39	43	46	188	316 (89 0)	$df=3$
Not stated	0	0	0	2	2 (0 6)	$p>0\ 05$
Total	47 (13 2)	47 (13 2)	55 (15 5)	206 (58 0)	355 (100 0)	
Income						
≤60 000 D kr	10	13	28	49	100 (28 2)	$\chi^2=17$ [illegible]
>60 000 D kr	36	33	26	153	248 (69 9)	$df=3$
Not stated	1	1	1	4	7 (1 9)	$p<0\ 001$
Total	47 (13 2)	47 (13 2)	55 (15 5)	206 (58 0)	355 (100 0)	
Residence						$\chi^2=1\ 6$
≤3 rooms	13	9	10	45	77 (21 7)	$df=3$
>3 rooms	34	38	45	161	278 (78 3)	$p>0\ 05$
Total	47 (13 2)	47 (13 2)	55 (15 5)	206 (58 0)	355 (100 0)	

Among elementary school children the analogous percentages were 15 7 and 16 5 The reasons for hospitalization of relatives were not explored

Among the preschool children five (2 2%) had experienced death in the near family Among elementary school children only one had lost a parent by death

Many of those life events which were related to the parents occupation also had a relatively high frequency Among wage earners with preschool children 14 9% of the fathers and 19 5% of the mothers had been unemployed in the year prior to admission of the child For 4–8% of the children there was information concerning the parents increased absence from home due to work Remarkably many mothers of preschool children (15 5%) had started working during the year prior to admission Changes in the parents financial status were as frequent as 30% among the parents of preschool children and 20% of the parents of elementary school children stated change in financial status

6 6% of preschool children and 3 9% of elementary school children had experienced divorce or separation of parents during the year prior to admission 17 7% of the children had or had had a single supporter Remarkably high frequencies of increased interpersonal conflicts were found 12% reported increase in number of arguments between parents Among preschool children the percentage of increase in number of arguments with parents or with peers was 5 the analogous percentages for elementary school children were 14 2 and 26 5

Correlations between selected social factors life events and diagnostic groups

As expected there were positive intercorrelations between the parents school education occupational education and income Lower income was among other things correlated to increased conflicts with and between parents and to increased conflict with peers There were also positive correlations between lower income and broken home hospitalization of parents and changes in parents financial status Unemployment of fathers was positively correlated with broken home and lower in-

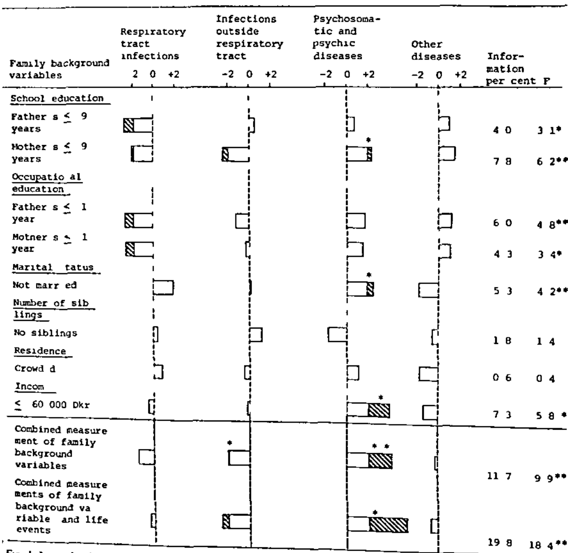


Fig 1 Normalized means for single and combined measurements of family background variables and life events for preschool children

** Significant on 1 o/oo level
* Significant on 1% level
Significant on 5% level

come and mothers unemployment was positively correlated to fathers unemployment

It is of special interest that psychosomatic diseases are positively correlated to low level of education and broken home and to events as hospitalization of siblings and increase in number of arguments with peers The psychosomatic diseases were negatively correlated with only child Consideration of correlation matrix might then suggest that an analysis necessarily must take into account the intercorrelations between social factors and life events

Combined measurements of social factors and life events by diagnostic groups

Preschool children s diagnostic groups are profiled in Fig 1 by selected family background variables The group of respiratory tract infections were characterized by higher level of education of the parents but the other parameters did not deviate significantly The

Table 2 *Diagnostic group by the parents marital status income and number of rooms in residence*

Parents marital status income and residence	Respiratory tract infections	Infections outside respiratory tracts	Psychosomatic and psychic diseases	Other diseases	Total number per cent	χ^2 test (corrected)
Marital status						
Not married	8	4	9	16	37 (10 4)	$\chi^2=5\ 9$
Married	39	43	46	188	316 (89 0)	$df=3$
Not stated	0	0	0	2	2 (0 6)	$p>0\ 05$
Total	47 (13 2)	47 (13 2)	55 (15 5)	206 (58 0)	355 (100 0)	
Income						
≤60 000 D kr	10	13	28	49	100 (28 2)	$\chi^2=17\ ?$
>60 000 D kr	36	33	26	153	248 (69 9)	$df=3$
Not stated	1	1	1	4	7 (1 9)	$p<0\ 001$
Total	47 (13 2)	47 (13 2)	55 (15 5)	206 (58 0)	355 (100 0)	
Residence						$\chi^2=1\ 6$
≤3 rooms	13	9	10	45	77 (21 7)	$df=3$
>3 rooms	34	38	45	161	278 (78 3)	$p>0\ 05$
Total	47 (13 2)	47 (13 2)	55 (15 5)	206 (58 0)	355 (100 0)	

Among elementary school children the analogous percentages were 15 7 and 16 5 The reasons for hospitalization of relatives were not explored

Among the preschool children five (2 2%) had experienced death in the near family Among elementary school children only one had lost a parent by death

Many of those life events which were related to the parents occupation also had a relatively high frequency Among wage earners with preschool children 14 9% of the fathers and 19 5% of the mothers had been unemployed in the year prior to admission of the child For 4–8% of the children there was information concerning the parents increased absence from home due to work Remarkably many mothers of preschool children (15 5%) had started working during the year prior to admission Changes in the parents financial status were as frequent as 30% among the parents of preschool children and 20% of the parents of elementary school children stated change in financial status

6 6% of preschool children and 3 9% of elementary school children had experienced divorce or separation of parents during the year prior to admission 17 7% of the children had or had had a single supporter Remarkably high frequencies of increased interpersonal conflicts were found 12% reported increase in number of arguments between parents Among preschool children the percentage of increase in number of arguments with parents or with peers was 5 the analogous percentages for elementary school children were 14 2 and 26 5

Correlations between selected social factors life events and diagnostic groups

As expected there were positive intercorrelations between the parents school education occupational education and income Lower income was among other things correlated to increased conflicts with and between parents and to increased conflict with peers There were also positive correlations between lower income and broken home hospitalization of parents and changes in parents financial status Unemployment of fathers was positively correlated with broken home and lower in

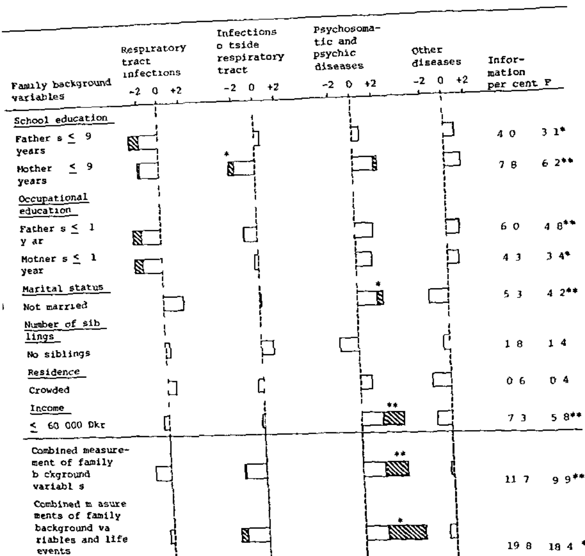


Fig 1 Normalized means for single and combined meas urements of family background variables and life events *for preschool children*

come and mothers unemployment was posi tively correlated to fathers unemployment

It is of special interest that psychosomatic diseases are positively correlated to low level of education and broken home and to events as hospitalization of siblings and increase in number of arguments with peers The psychosomatic diseases were negatively cor related with only child Consideration of correlation matrix might then suggest that an analysis necessarily must take into account the intercorrelations between social factors and life events

Combined measurements of social factors and life events by diagnostic groups

Preschool children s diagnostic groups are profiled in Fig 1 by selected family back ground variables The group of respiratory tract infections were characterized by higher level of education of the parents but the other parameters did not deviate significantly The

Table 2 *Diagnostic group by the parents' marital status, income and number of rooms in residence*

Parents' marital status, income and residence	Respiratory tract infections	Infections outside respiratory tracts	Psychosomatic and psychic diseases	Other diseases	Total number per cent	χ^2 test (corrected)
Marital status						
Not married	8	4	9	16	37 (10.4)	$\chi^2=5.9$
Married	39	43	46	188	316 (89.0)	$df=3$
Not stated	0	0	0	2	2 (0.6)	$p>0.05$
Total	47 (13.2)	47 (13.2)	55 (15.5)	206 (58.0)	355 (100.0)	
Income						
≤60 000 D kr	10	13	28	49	100 (28.2)	$\chi^2=17.$[illegible]
>60 000 D kr	36	33	26	153	248 (69.9)	$df=3$
Not stated	1	1	1	4	7 (1.9)	$p<0.001$
Total	47 (13.2)	47 (13.2)	55 (15.5)	206 (58.0)	355 (100.0)	
Residence						$\chi^2=1.6$
≤3 rooms	13	9	10	45	77 (21.7)	$df=3$
>3 rooms	34	38	45	161	278 (78.3)	$p>0.05$
Total	47 (13.2)	47 (13.2)	55 (15.5)	206 (58.0)	355 (100.0)	

Among elementary school children the analogous percentages were 15.7 and 16.5. The reasons for hospitalization of relatives were not explored.

Among the preschool children five (2.2%) had experienced death in the near family. Among elementary school children only one had lost a parent by death.

Many of those life events, which were related to the parents' occupation, also had a relatively high frequency. Among wage earners with preschool children 14.9% of the fathers and 19.5% of the mothers had been unemployed in the year prior to admission of the child. For 4–8% of the children there was information concerning the parents' increased absence from home due to work. Remarkably many mothers of preschool children (15.5%) had started working during the year prior to admission. Changes in the parents' financial status were as frequent as 30% among the parents of preschool children, and 20% of the parents of elementary school children stated change in financial status.

6.6% of preschool children and 3.9% of elementary school children had experienced divorce or separation of parents during the year prior to admission. 17.7% of the children had or had had a single supporter. Remarkably high frequencies of increased interpersonal conflicts were found. 12% reported increase in number of arguments between parents. Among preschool children the percentage of increase in number of arguments with parents or with peers was 5, the analogous percentages for elementary school children were 14.2 and 26.5.

Correlations between selected social factors, life events and diagnostic groups

As expected, there were positive intercorrelations between the parents' school education, occupational education and income. Lower income was among other things correlated to increased conflicts with and between parents and to increased conflict with peers. There were also positive correlations between lower income and broken home, hospitalization of parents and changes in parents' financial status. Unemployment of fathers was positively correlated with broken home and lower in-

only yield moderate information in this age group By combining the measurements for family background variables it was found that the tendency was the same as in the group of preschool children but not so pronounced It is essential to note however that life events did yield significant additional information and that this was related to a further significant deviation for the group of children with psychosomatic and psychic diseases

It is now natural to ask how the selected life events are distributed on the diagnoses in the two age groups

Preschool children s diagnostic groups are profiled in Fig 2 by selected life events The individual life events showed no clear tendency but there were significantly more children with respiratory tract infections who had had increased number of arguments with parents The combined measurement for life events revealed high values among respiratory tract infections as well as psychosomatic and psychic diseases most pronounced for the latter diagnostic group

Elementary school children s diagnostic groups were profiled in the same way by selected life events More life events show significant deviations Especially conflict with peers and hospitalization of siblings should be emphasized The maximal information due to life events was significantly greater in this age group than among preschool children The combined measurement for life events shows strong positive significant deviation in the group of psychosomatic and psychic diseases

DISCUSSION

The investigation has a relatively large non response group However there was no significant difference between composition of the response group and the non response group

The questions which describe the social background were relatively simple and a further simplification occurred during data processing For those reasons such a design can only point to statistical associations between the independent variables but beyond this a statistical estimation of the relative weight of these is possible In other words one cannot demonstrate causal relations but only associations with possible etiological determinants

The classification into diagnostic groups is relatively coarse but nevertheless there was a great deal of clinical homogeneity within groups which makes meaningful explanations possible The remainder group did not deviate significantly in any respect

It was characteristic that the selected social factors had greatest significance among preschool children but the diagnostic groups for elementary school children tended towards the same type of profile

That negative social factors such as low level of education and relatively low income were frequent among children with psychosomatic and/or psychic diseases and that the opposite was the case within the group of respiratory tract infections is an observation which is contrary to earlier investigations in social medicine (2)

This suggests that the significance of social factors has changed in recent time As an example residence might be mentioned Nearly 3/4 of the children lived in residences with more than 3 rooms and only 1/10 lived in a crowded residence Neither number of rooms nor crowded residence seemed to be of significance in differing between groups This has naturally something to do with these essential improvements in the technical and hygienic condition of housing which have taken place during the last generation That does not mean that a poor residence cannot be a health risk but at present relatively few children grow up in unhealthy residences

The absolute frequency of selected life events were relatively high but only for a few of the selected life events was there a significant association with diagnostic groups The combined measurement for life events however showed a positive significant deviation

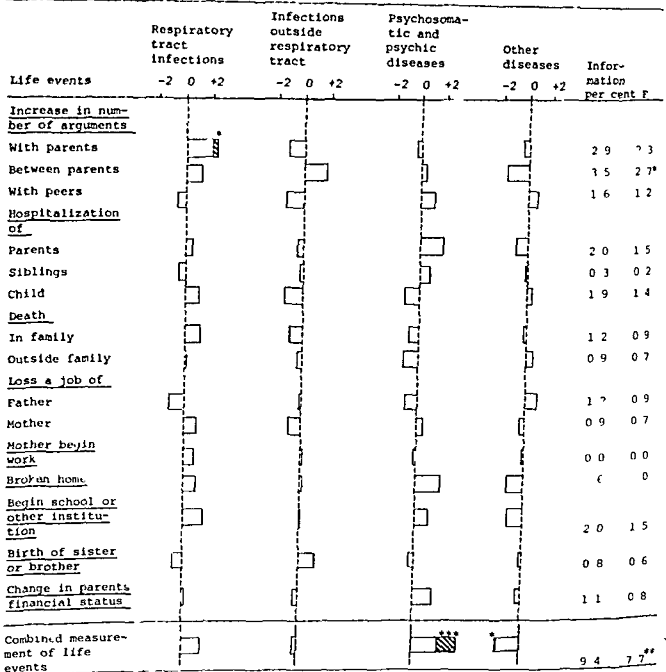


Fig. 2 Normalized means for single and combined measurements of life events for preschool children

group of psychosomatic and psychic diseases was characterized by low level of education, not married and low income.

The selected family background variables were employed to create a combined measurement which took into account the correlations between the independent variables. As shown in Fig. 1 the group of psychosomatic and psychic diseases deviated significantly.

A new combined measurement including family background variables and life events increased the information a great deal. The additional information was essentially due to the further significant deviation of the group of psychosomatic and psychic diseases.

Elementary school children's diagnostic groups were profiled in the same way. It is characteristic that the individual social factors

only yield moderate information in this age group By combining the measurements for family background variables it was found that the tendency was the same as in the group of preschool children but not so pronounced It is essential to note however that life events did yield significant additional information and that this was related to a further significant deviation for the group of children with psychosomatic and psychic diseases

It is now natural to ask how the selected life events are distributed on the diagnoses in the two age groups

Preschool children s diagnostic groups are profiled in Fig 2 by selected life events The individual life events showed no clear tendency but there were significantly more children with respiratory tract infections who had had increased number of arguments with *parents* The combined measurement for life events revealed high values among respiratory tract infections as well as psychosomatic and psychic diseases most pronounced for the latter diagnostic group

Elementary school children s diagnostic groups were profiled in the same way by selected life events More life events show significant deviations Especially conflict with peers and hospitalization of siblings should be emphasized The maximal information due to life events was significantly greater in this age group than among preschool children The combined measurement for life events shows strong positive significant deviation in the group of psychosomatic and psychic diseases

DISCUSSION

The investigation has a relatively large *non* response group However there was no significant difference between composition of the response group and the non response group

The questions which describe the social background were relatively simple and a further simplification occurred during data processing For those reasons such a design can only point to statistical associations between the independent variables but beyond this a statistical estimation of the relative weight of these is possible In other words one cannot demonstrate causal relations but only associations with possible etiological determinants

The classification into diagnostic groups is relatively coarse but nevertheless there was a great deal of clinical homogeneity within groups which makes meaningful explanations possible The remainder group did not deviate significantly in any respect

It was characteristic that the selected social factors had greatest significance among pre school children but the diagnostic groups for elementary school children tended towards the same type of profile

That negative social factors such as low level of education and relatively low income were frequent among children with psycho somatic and/or psychic diseases and that the opposite was the case within the group of *respiratory* tract infections is an observation which is contrary to earlier investigations in social medicine (2)

This suggests that the significance of social factors has changed in recent time As an example residence might be mentioned Nearly 3/4 of the children lived in residences with more than 3 rooms and only 1/10 lived in a crowded residence Neither number of rooms nor crowded *residence* seemed to be of significance in differing between groups This has naturally something to do with these essential improvements in the technical and *hygienic* condition of housing which have taken place during the last generation That does not mean that a poor residence cannot be a health risk but at present relatively few children grow up in unhealthy residences

The absolute frequency of selected *life* events were relatively high but only for a few of the selected life events was there a significant association with diagnostic groups The combined measurement for life events however *showed* a positive significant deviation

for the group of psychosomatic and psychic diseases

It was remarkable that there was also a tendency towards large numbers of life events among preschool children who had a respiratory tract infection as main diagnosis These children were nearly all admitted as emergencies

However this investigation cannot clarify whether large numbers of life events had true etiologic significance or only influenced the indication for admission to hospital

The combined measurement was computed on the background of the intercorrelations by applying the method of appropriate scoring The applied method differs from life event/life change unit (LCU) procedure where pre assigned *life event scores usually are applied*

With a few exceptions (7) life event/LCU research did not take into account the possible intercorrelations between life events and social factors Noticeable correlations between social factors and life events were found in this study On this basis it can hardly be doubted that life events should not be considered alone, but must be evaluated in connection with the family background variables

It was not contrary to expectation that the *combined measurement for family background* variables and life events were strongly associated with psychosomatic and psychic diseases but it was remarkable that the additional information from life events appeared so strongly The results suggest that stressful life events in combination with negative social factors increases the risk of developing a psychosomatic or psychic disease of such a severity that the child has to be admitted to hospital

Consequences of the results

The new aspect to which this study has contributed is to clarify the relative weight of family background variables and life events for various disease categories

As stressful life events also frequently occur prior to admission of children with some somatic diseases there is a consequent plea for a detailed recording of the social history of the individual child

The purpose of this is to identify those children whose symptoms or disease are better understood within a social or sociopsychological framework

Furthermore the results of the investigation might have implications for the preventive work with an understanding of those changes which seem to have taken place in important social conditions and the role which stressful life events might have as a precipitant of disease

ACKNOWLEDGEMENT

This study was made possible through grant from the Danish Medical Research Council

REFERENCES

1 Apley J & McKeith R *The child and his symptoms* Blackwell Scientific Publications Oxford 1968
2 Christensen V *Boligforhold og børnesygelighed* Munksgaard Copenhagen 1956
3 Christensen V Trivselsproblemer og sygdomsreaktioner i førskolealderen *Ugeskr Laeger 136* 1897 1974
4 Coddington R D The significance of life events as etiologic factors in the diseases of children *J Psychosom Res 16* 7 1972
5 Coddington R D The significance of life events as etiologic factors in the diseases of children II *J Psychosom Res 16* 20 1972
6 *Folke og boligtællingen* Danmarks Statistik Copenhagen 1973
7 Gersten J C Langner T S Eisenberg J G & Simcha Fugan O An evaluation of the etiologic role of stressful life change events in psychological disorders *J Health Soc Behav 18* 228 1977
8 Helsinki II Declaration *Ugeskr Laeger 138* 399 1976
9 Robertson J *Young children in hospital* Tavistock Publications Baltimore 1958
10 Robins B N *Deviant children grow up* The Williams & Wilkins Company Baltimore 1966
11 Rosberg G Social and psychological factors influencing hospital admission of children *Acta Paediatr Scand* Suppl 196 1969
12 Rutter M Psychosocial disorders in childhood and their outcome in adult life *J R Coll Physicians London 4* 211 1970
13 Stocking M Rothney W Grosser G & Goodwin R Psychopathology in the paediatric hospital Implication for community health *Am J Public Health 62* 551 1972

14 Straus P Coffard N Marzo Weyl S & Lenoir M *L hospitalisation des enfants Une etude de pediatrie sociale dans l'agglomeration parisienne* Monographie de l Institut National d Hygiene No 73 Paris 1961
15 Aagaard J Psykosocial belastning og sygdom hos born Metodediskussion *Ugeskr Laeger 139* 2961 1977
16 Aagaard J & Bro P Psykosocial belastning og syg dom hos born Evaluering af pilot materiale *Ugeskr Laeger 139* 2966 1977
17 Aagaard J *Admission to hospital An analysis of the social history of children admitted to a paediatric department* Institute of Social Medicine University of Aarhus No 18 1978

Submitted Aug 21 1978
Accepted Oct 23 1978

(J A) Institute of Social Medicine
University of Aarhus
Vesterbro Torv 1-3 6
DK 8000 Århus C
Denmark

Acta Pædiatr Scand 68 541–545 1979

SCREENING FOR HYPERLIPOPROTEINEMIA IN 10 000 DANISH NEWBORNS

Follow up Studies in 522 Children with Elevated Cord Serum VLDL–LDL–Cholesterol

GUNNAR E ANDERSEN PER LOUS and BENT FRIIS HANSEN

From the Neonatal Department Rigshospitalet and the Department of Clinical Chemistry Bispebjerg Hospital University of Copenhagen Copenhagen Denmark

ABSTRACT Andersen G E Lous P and Friis-Hansen B (Neonatal Department Rigshospitalet and Department of Clinical Chemistry Bispebjerg Hospital Copenhagen Denmark) Screening for hyperlipoproteinemia in 10 000 Danish newborns Follow up studies in 522 children with elevated cord serum VLDL–LDL–cholesterol Acta Paediatr Scand 68 541 1979 —Among 10 440 newborns 522 with upper 5 percentile values for very low low density lipoprotein cholesterol in cord serum were selected for follow up studies Follow up was possible in 446 of these 522 families (85%) and familial hyper cholesterolemia (FH) was diagnosed in 11 In 273 of the 522 children serum lipids were determined between the ages of 1 and 2 years and were now found to be normal except in the 11 children with FH Furthermore the serum lipids were compared in subgroups of these 273 children divided according to obstetric complications (i e low birth weight perinatal asphyxia and antepartum betamethasone treatment) which may cause a rise in serum lipids at birth No differences were found between these subgroups at the age of 1–2 years

KEY WORDS Cholesterol triglyceride lipoproteins children screening familial hyper cholesterolemia

Recently (1) we have shown that newborns with elevated cord serum VLDL–LDL–C can be identified by a turbidimetric estimation of cord serum VLDL–LDL and that besides FH also prematurity perinatal asphyxia and antepartum betamethasone may cause a rise in cord serum VLDL–LDL–C

In this paper we present data to elucidate 1) how many children with upper 5 percentile values for cord serum VLDL–LDL–C have FH 2) if the serum lipids will later on become normal or remain elevated in children with elevated serum VLDL–LDL–C at birth and who do not have FH and 3) if the serum lipids in later childhood are the same or different in subgroups of children with elevated serum VLDL–LDL–C at birth and who differed with respect to gestational age perinatal asphyxia and betamethasone treatment

MATERIALS

Between September 1975 and February 1977 cord blood was obtained as described earlier (2) from 10 440 infants consecutively born in the 6 major obstetric departments in Copenhagen (Sct Joseph s Frederiksberg Oresund Glostrup and Gentofte Hospital and the Rigshospitalet) Cord serum VLDL–LDL was measured by the turbidimetric method (3) and 1879 of the 10 440 (18%) with the highest VLDL–LDL values were selected for a more extended lipid and lipoprotein determination Of these 1 879 newborns 522 (5%) with the highest VLDL–LDL–C levels were subsequently selected for follow up studies to ascertain whether they belonged to families with FH

Abbreviations FH = familial hypercholesterolemia AGA = appropriate for gestational age SGA = small for gestational age T C = total cholesterol TG = triglyceride VLDL = very low density lipoproteins LDL = low density lipoproteins HDL = high density lipoproteins VLDL–LDL = VLDL+LDL determined by turbidimetry VLDL–LDL–C = VLDL–cholesterol + LDL–cholesterol determined enzymatically after CaCl heparin precipitation HDL–C = HDL–cholesterol calculated as the difference (T–C) – (VLDL–LDL–C)

Acta Pædiatr Scand 68 541–545 1979

SCREENING FOR HYPERLIPOPROTEINEMIA IN 10 000 DANISH NEWBORNS

Follow up Studies in 522 Children with Elevated Cord Serum VLDL–LDL–Cholesterol

GUNNAR E ANDERSEN PER LOUS and BENT FRIIS HANSEN

From the Neonatal Department Rigshospitalet and the Department of Clinical Chemistry Bispebjerg Hospital University of Copenhagen Copenhagen Denmark

ABSTRACT Andersen G E Lous P and Friis-Hansen B (Neonatal Department Rigshospitalet and Department of Clinical Chemistry Bispebjerg Hospital Copenhagen Denmark) Screening for hyperlipoproteinemia in 10 000 Danish newborns Follow up studies in 522 children with elevated cord serum VLDL–LDL–cholesterol Acta Paediatr Scand 68 541 1979 —Among 10 440 newborns 522 with upper 5 percentile values for very low low density lipoprotein cholesterol in cord serum were selected for follow up studies Follow up was possible in 446 of these 522 families (85%) and familial hyper cholesterolemia (FH) was diagnosed in 11 In 273 of the 522 children serum lipids were determined between the ages of 1 and 2 years and were now found to be normal except in the 11 children with FH Furthermore the serum lipids were compared in subgroups of these 273 children divided according to obstetric complications (i e low birth weight perinatal asphyxia and antepartum betamethasone treatment) which may cause a rise in serum lipids at birth No differences were found between these subgroups at the age of 1–2 years

KEY WORDS Cholesterol triglyceride lipoproteins children screening familial hyper cholesterolemia

Recently (1) we have shown that newborns with elevated cord serum VLDL–LDL–C can be identified by a turbidimetric estimation of cord serum VLDL–LDL and that besides FH also prematurity perinatal asphyxia and antepartum betamethasone may cause a rise in cord serum VLDL–LDL–C

In this paper we present data to elucidate 1) how many children with upper 5 percentile values for cord serum VLDL–LDL–C have FH 2) if the serum lipids will later on become normal or remain elevated in children with elevated serum VLDL–LDL–C at birth and who do not have FH and 3) if the serum lipids in later childhood are the same or different in subgroups of children with elevated serum VLDL–LDL–C at birth and who differed with respect to gestational age perinatal asphyxia and betamethasone treatment

MATERIALS

Between September 1975 and February 1977 cord blood was obtained as described earlier ( ) from 10 440 infants consecutively born in the 6 major obstetric departments in Copenhagen (Sct Joseph s Fredenksberg Öresund Glostrup and Gentofte Hospital and the Rigshospitalet) Cord serum VLDL–LDL was measured by the turbidimetric method (3) and 1879 of the 10 440 (18%) with the highest VLDL–LDL values were selected for a more extended lipid and lipoprotein determination Of these 1879 newborns 522 (5%) with the highest VLDL–LDL–C levels were subsequently selected for follow up studies to ascertain whether they belonged to families with FH

Abbreviations FH = familial hypercholesterolemia AGA = appropriate for gestational age SGA = small for gestational age T C = total cholesterol TG = triglyceride VLDL = very low density lipoproteins LDL = low density lipoproteins HDL = high density lipoproteins VLDL–LDL = VLDL+LDL determined by turbidimetry VLDL–LDL–C = VLDL–cholesterol + LDL–cholesterol determined enzymatically after CaCl heparin precipitation HDL–C = HDL–cholesterol calculated as the difference (T–C) – (VLDL–LDL–C)

Table 1 *Lipid and lipoprotein–cholesterol values (mmol/l) in 11 pairs of parent–child with familial hypercholesterolemia (FH)*

Kindred	Age (years)	T–C (mmol/l)	VLDL–LDL–C (mmol/l)	LDL–C (mmol/l)	HDL–C (mmol/l)	TG (mmol/l)
1 II 5	31	8 54	5 41	5 ?5	3 13	0 84
1 III 5	At birth	? 87	1 58		1 _4	0 61
1 III 5	1°/	6 43	5 17	4 61	1 ?6	1 81
II 1	3?	8 89	6 35	5 67	2 54	1 79
? III 4	At birth	11	1 35		0 76	0 73
? III 4	1 / 2	7 16	5 _?	4 72	1 94	1 ?5
3 II 6	?3	11 06	9 45	8 96	1 61	0 60
3 III 4	At birth	4 6	1 69		2 57	0 90
3 III 4	1	7 5?	4 78	4 55	? 74	0 93
4 II 1	9	11 16	9 43	8 88	1 73	1 3?
4 III 1	At birth	? 96	1 61		1 35	0 59
4 III 1	1 /12	8 45	6 78	6 47	1 67	0 90
5 II ?	?1	9 69	6 88	6 61	? 81	0 97
5 III 3	At birth	3 79	1 77		2 0?	0 47
5 III 3	/	7 ?6	4 24	4 00	3 0?	0 73
6 II 7	?9	7 80	5 41	4 45	? 39	2 55
6 III 6	At birth	3 15	1 45		1 70	0 53
6 III 6	1 / 2	6 53	4 46	4 0?	? 07	1 36
7 I 9	8	8 0	5 56	4 73	? 64	1 69
7 II 15	At birth	1 90	1 45		0 45	0 64
7 II 15	/	7 11	5 31	5 01	1 80	1 36
8 II ?	38	9 1?	6 03	5 9?	3 09	0 63
8 III 4	At birth	3 89	? 45		1 44	0 64
8 III 4	1	7 8	5 6	5 ?6	1 66	1 57
9 II	19	9 67	7 1?	6 85	? 55	0 59
9 III 1	At birth	? 8	1 30		0 98	0 5?
9 III 1	1°/ 2	7 35	4 9?	4 71	2 43	0 79
10 III 5	31	9 51	7 41	7 17	? 10	0 74
10 IV 6	At birth	? 4?	1 33		1 09	0 30
10 IV 6	1 /12	7 21	5 16	5 00	05	0 78
11 II 1	4	7 41	4 ?8	4 18	3 13	0 59
11 III 1	At birth	74	1 54		1 ?0	0 83
11 III 1	? / 2	6 93	5 05	4 73	1 88	1 03

f On type II diet

FH is here defined as an autosomal dominantly in herited disea e with a three generation vertical trans mission of hypercholesterolemia (>95th sex and age adjusted value for serum T–C and/or LDL–C in nor mal) Su pici n of FH arises by the finding of hyper cholesterolemia in one of the parents o in the child after age 1 (4) and so serum lipids were examined in either the parents and/ r the child

In 76 cases (15°) no follow up was possible in either the parents or the child 2 of these 76 children had died before age 1 and it was decided not to study their parents The remaining 54 families were not interested in participating

In ?97 of the 5? families venous blood samples were taken from both parents after a 1 -hour fast and in the mothers at least 3 months after birth In ?98 families venous blood samples were taken from ?98 children after age 1 (?73 1– years old _5 over years) after an 8-hour fast This meant that in 149 families therefore both parents and the child were examined in 148 families only the parents and in 149 families only the child was examined 1 serum T–C was found to be elevated in either the parents or the child (>95th sex and age adjusted percentile values for normal Danes (5)) an ex tended serum lipid and lipoprotein determination was made at least three times and all available family members were asked to join the study and their serum lipids and lipoproteins were determined

METHODS

The methods are the ones already described (?) except that at follow-up in parents and children serum T–C was measured enzymatically on a Greiner Selective Analyzer II as described by Bomer & Klose (6)

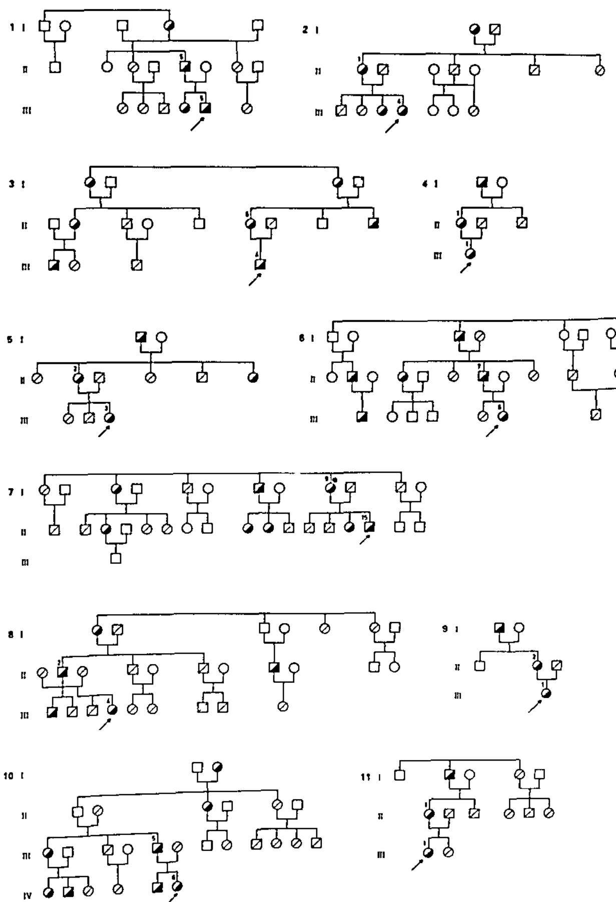


Fig. 1. The kindreds □ ○ Not sampled (deceased or abroad) ⊘ ⊘ normal ◪ ◑ type IIa ⊞ ⊕ died from premature myocardial infarction xanthomas ↗ included in cord blood screening

cord serum VLDL-LDL-C and since a yet unknown number of children with FH may under certain circumstances be born with a normal cord serum VLDL-LDL-C level as shown earlier (1)

A minimum estimate however is possible since among 10 440 consecutively born infants we have found 11 with a three generation vertical transmission of hypercholesterolemia which is generally accepted as an accurate criterion of FH (4) Thus a minimal incidence seems to be 0 11% which is close to 0 17% found by Tsang et al (9) and 0 10% reported by Andersen & Friis Hansen (1) using the same diagnostic criteria This makes FH the single most common inborn error of metabolism reported in Denmark so far

Children with elevated cord serum VLDL-LDL-C irrespective of the genesis of this elevation (prematurity perinatal asphyxia betamethasone treatment) with the one important exception of FH had normal serum T-C and LDL-C at follow up between the ages of 1 and 2 years The higher levels of serum TG and VLDL-C are probably explained by a fast of only 8 hours in these children as opposed to 10-12 hours in the reference group This is an agreement with the findings of Boulton et al (10) who reinvestigated 49 of 81 children with elevated cord serum LDL-C At 4-12 months of age their serum T-C and LDL-C values were the same as in a control group Hardell (11) reinvestigated an unreported number of children with elevated cord serum VLDL-LDL-C during their second year of life In boys the same serum lipid levels were found as in a reference group whereas the girls had slightly higher T-C VLDL-LDL-C and HDL-C values

In conclusion the present study has shown that it was possible in at least 0 11% to make the diagnosis FH by a follow up study of the families of children who at birth had elevated serum VLDL-LDL-C However the vast majority of children with elevated VLDL-LDL-C values at birth have normal serum lipids at follow up

ACKNOWLEDGEMENTS

This study was supported by grants from the Danish Heart Foundation and the Dagmar Marshall Fund

REFERENCES

1 Andersen G E Lous P & Friis Hansen B Hyperlipoproteinemia in newborn infants A study of 1025 families *Acta Paediatr Scand* in press

Andersen G E & Friis Hansen B Cord serum lipid and lipoprotein-cholesterol values in normal and betamethasone treated newborns of varying gestational age *Acta Paediatr Scand 66* 355 1977

3 Andersen G E & Gry Nielsen H Neonatal screening for hyperlipoproteinemia Methods for direct estimation of cord serum VLDL+LDL *Clin Chim Acta 66* 29 1976

4 Fredrickson D S Goldstein J L & Brown M S The familial hyperlipoproteinemias *In* J B Stanbury J B Wyngaarden & D S Fredrickson (eds) *The metabolic basis of inherited disease* McGraw Hill New York 1978 p 604

5 Andersen G E & Friis Hansen B Neonatal diagnosis of familial type II hyperlipoproteinemia *Pediatrics 57* 214 1976

6 Borner K & Klose S Enzymatische Bestimmung des Gesamtcholesterins mit dem Greiner Selective Analyzer (GSA II) *J Clin Chem Clin Biochem 15* 1 1 1977

7 Sigel S *In* S Siegel (ed) *Nonparametric statistics for the behavioral sciences* McGraw Hill New York 1956 pp 116 and 203

8 Andersen G E Lifschitz C & Friis Hansen B Dietary habits and serum lipids during first 4 years of life A study of 95 Danish children *Acta Pædiatr Scand* in press

9 Tsang R C Glueck C J Fallat R W & Mellies M Neonatal familial hypercholesterolemia *Am J Dis Child 129* 83 1975

10 Personal communication

11 Hardell L I *Serum lipids and lipoproteins at birth and in early childhood* Thesis Uppsala 1978

Submitted Oct 1 1978
Accepted Dec 1 1978

(G E A) Neonatal Department GN 50 4
Rigshospitalet
Blegdamsvej 9
DK 100 Copenhagen
Denmark

Table 2. *Percentile values for serum lipids and lipoproteins in 265 children at follow-up between age 1–2 years*

Maturity at birth	Serum lipid values (mmol/l) 5	10	50	90	95
I AGA ≥37<42 weeks ($n=190$)					
T–C	3.48	3.69	4.75	5.77	6.08
VLDL–LDL–C	2.04	2.25	3.04	3.85	4.21
HDL–C	1.08	1.20	1.64	2.15	2.36
TG	0.53	0.59	0.84	1.42	1.70
II AGA <37 weeks ($n=47$)					
T–C	3.54	3.69	4.63	5.64	6.14
VLDL–LDL–C	2.15	2.31	3.01	3.82	4.40
HDL–C	1.05	1.14	1.63	2.17	2.38
TG	0.49	0.58	0.87	2.01	2.10
III SGA ≥33<42 weeks ($n=28$)					
T–C	3.40	3.50	4.65	5.68	5.85
VLDL–LDL–C	2.09	2.30	3.16	3.85	3.88
HDL–C	0.89	1.06	1.58	1.91	2.32
TG	0.45	0.52	0.83	1.74	1.80

Two cholesterol standards (K 77 and Pathonorm) were included in over 250 separate runs and the coefficients of variation were 3.1 and 4.0 resp.

The non-parametric statistical methods used are the same as the ones described by Siegel (7).

RESULTS

Among the 446 families available for follow-up studies, FH was found in 11. The 11 kindreds are depicted in Fig. 1 and the corresponding lipid and lipoprotein values in parent–child are given in Table 1. None of the 11 children had suffered perinatal asphyxia nor been given betamethasone. They were all delivered at term and were AGA. In all 11 parents common causes of secondary hyperlipemia were ruled out by repeated findings of normal glucose tolerance test, normal liver, thyroid and kidney function tests and normal immunoglobulins. Only one parent (11 II 1), a 24-year-old woman, was known to have FH and had been treated with a type II diet, but no drugs for 1 ½ years at the time we investigated the serum lipids of her daughter.

In Table 2 the percentile values are given for the serum lipids in 265 of the 273 children reinvestigated between the ages of 1 and 2 years. The values of 8 postmature newborns are left out.

Within each of the three groups (I mature, II premature and III small for date newborns) the lipid data of the four subgroups 1) no asphyxia, no betamethasone, 2) asphyxia, no betamethasone, 3) no asphyxia, betamethasone, 4) asphyxia, betamethasone have been pooled since no differences ($p>0.05$) were found between 1, 1 and 2 or between 1, 3 and 4.

A comparison of the lipid values in groups I, II and III did not show any differences ($p>0.05$). Neither were there any differences between the lipid values in boys and girls ($p>0.05$). Furthermore, the lipid values in groups I, II and III were compared with the lipid values of a reference group of 64 normal Danish children aged 3–4 years (8). Serum T–C and LDL–C levels were similar in the two groups ($p>0.05$). Serum TG and VLDL–C levels, however, were higher ($p<0.001$) in the children aged 1–2 years.

DISCUSSION

The present study does not allow an accurate calculation of the incidence of FH in Danish children since follow-up studies were only possible in 85 % of the newborns with elevated

Acta Pædiatr Scand 68 547–552 1979

SMOKING DURING PREGNANCY—EFFECTS ON THE FETUS AND ON THIOCYANATE LEVELS IN MOTHER AND BABY

A MEBERG H SANDE O P FOSS and J T STENWIG

From the Department of Paediatrics the Department of Obstetrics and Gynecology the Department of Clinical Chemistry and the Department of Pathology Ullevål Hospital Oslo Norway

ABSTRACT Meberg A Sande H Foss O P and Stenwig J T (Departments of Paediatrics Obstetrics and Gynecology Clinical Chemistry and Pathology Ullevål Hospital Oslo Norway) Smoking during pregnancy—effects on the fetus and on thiocyanate levels in mother and baby Acta Paediatr Scand 68 547 1979.—Twenty-eight mothers smoking 10–20 cigarettes daily during pregnancy had significantly higher serum thiocyanate concentrations at delivery compared to 25 non smoking controls The thiocyanate levels were positively correlated to cigarette consumption and inversely correlated to the birth weights of the infants A highly significant correlation existed between serum thiocyanate levels of the mother and umbilical cord serum thiocyanate levels reflecting a nearly complete equilibration The thiocyanate concentrations in human milk on the 4th day after delivery were considerably lower than the serum concentrations and no correlation existed between serum and milk concentrations The infants of smoking mothers had significantly decreased weight and length at birth compared to infants of non smokers Birth weights were 3344±434 g and 3670±504 g respectively ($p<0.05$) and lengths 49 8±1 7 cm and 51±1 6 cm respectively ($p<0.05$) No differences were found between smokers and non smokers in placental and umbilical cord histology and umbilical cord artery medial area It is concluded that serum thiocyanate concentration in smokers may be used as an objective measure for smoke exposure and that maternal cigarette smoking acts as an exogenous factor which interferes with intrauterine development of the fetus in a dose related way

KEY WORDS Cigarette smoke pregnancy infants thiocyanate

Maternal cigarette smoking during pregnancy interferes with fetal development Smokers produce smaller babies (7 12 13 14 16) have a higher incidence of abortion (18) premature delivery (7 18) and perinatal mortality (7 13 18) than non smokers Long term effects of maternal smoking on physical growth (8) and intellectual development (9) are found Several factors are suggested to cause these effects on the fetus such as carbonmonoxide (6 17) and cyanide toxicity (1 16) Placental ultrastructural changes (2) as well as impaired placental blood flow (19) have also been described in smokers

In smoking individuals cyanide is absorbed from the smoke and detoxicated to thiocyanate in the body (5 20) Increasing serum thiocyanate levels have been found with increasing cigarette consumption (1 20)

The aim of the present investigation was to evaluate thiocyanate levels in mothers and their infants as an objective measure for smoke exposure and to investigate effects of maternal smoking on fetal size and on histology of the placenta and umbilical cord

MATERIALS AND METHODS

Fifty three mothers (28 smokers and 25 non-smokers) and their newborn infants were included in the series Consecutive smoking mothers were selected if they fulfilled the following criteria healthy women aged between 20 and 30 years in their first or second uncomplicated pregnancy a normal delivery at 39–42 weeks of gestation and a cigarette consumption estimated by the women themselves to be 10 15 or 20 cigarettes daily for the whole pregnancy The non smoking mothers selected

Acta Pædiatr Scand 68 547-552 1979

SMOKING DURING PREGNANCY—EFFECTS ON THE FETUS AND ON THIOCYANATE LEVELS IN MOTHER AND BABY

A MEBERG H SANDE O P FOSS and J T STENWIG

From the Department of Paediatrics the Department of Obstetrics and Gynecology the Department of Clinical Chemistry and the Department of Pathology Ullevål Hospital Oslo Norway

ABSTRACT Meberg A Sande H Foss O P and Stenwig J T (Departments of Paediatrics Obstetrics and Gynecology Clinical Chemistry and Pathology Ullevål Hospital Oslo Norway) Smoking during pregnancy—effects on the fetus and on thiocyanate levels in mother and baby Acta Paediatr Scand 68 547 1979 —Twenty-eight mothers smoking 10-20 cigarettes daily during pregnancy had significantly higher serum thiocyanate concentrations at delivery compared to 25 non-smoking controls The thiocyanate levels were positively correlated to cigarette consumption and inversely correlated to the birth weights of the infants A highly significant correlation existed between serum thiocyanate levels of the mother and umbilical cord serum thiocyanate levels reflecting a nearly complete equilibration The thiocyanate concentrations in human milk on the 4th day after delivery were considerably lower than the serum concentrations and no correlation existed between serum and milk concentrations The infants of smoking mothers had significantly decreased weight and length at birth compared to infants of non-smokers Birth weights were 3344±434 g and 3620±504 g respectively ($p<0.05$) and lengths 49 8±1 7 cm and 51±1 6 cm respectively ($p<0.05$) No differences were found between smokers and non smokers in placental and umbilical cord histology and umbilical cord artery medial area It is concluded that serum thiocyanate concentration in smokers may be used as an objective measure for smoke exposure and that maternal cigarette smoking acts as an exogenous factor which interferes with intrauterine development of the fetus in a dose related way

KEY WORDS Cigarette smoke pregnancy infants thiocyanate

Maternal cigarette smoking during pregnancy interferes with fetal development Smokers produce smaller babies (7 12 13 14 16) have a higher incidence of abortion (18) premature delivery (7 18) and perinatal mortality (7 13 18) than non smokers Long term effects of maternal smoking on physical growth (8) and intellectual development (9) are found Several factors are suggested to cause these effects on the fetus such as carbonmonoxide (6 17) and cyanide toxicity (1 16) Placental ultrastructural changes (2) as well as impaired placental blood flow (19) have also been described in smokers

In smoking individuals cyanide is absorbed from the smoke and detoxicated to thiocyanate in the body (5 20) Increasing serum thiocyanate levels have been found with increasing cigarette consumption (1 20)

The aim of the present investigation was to evaluate thiocyanate levels in mothers and their infants as an objective measure for smoke exposure and to investigate effects of maternal smoking on fetal size and on histology of the placenta and umbilical cord

MATERIALS AND METHODS

Fifty three mothers (28 smokers and 25 non-smokers) and their newborn infants were included in the series Consecutive smoking mothers were selected if they fulfilled the following criteria healthy women aged between 20 and 30 years in their first or second uncomplicated pregnancy a normal delivery at 39-42 weeks of gestation and a cigarette consumption estimated by the women themselves to be 10 15 or 20 cigarettes daily for the whole pregnancy The non smoking mothers selected

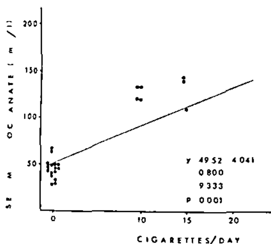


Fig 1 Relation between serum thiocyanate concentrations at delivery in 51 women and their smoking level during pregnancy

Blocks were taken from the cord membranes central and peripheral placental areas and processed in a routine fashion In addition 2–3 cm long pieces of cord were taken 10 cm from the placental insertion and fixed in buffered 4% formalin (pH 7 4) Transverse sections were made and stained by the Verhoff–van Gieson method to visualize the elastic lamina and the medial smooth muscle The arteries were photographed through a microscope In those cut at a right angle and with complete obliteration of the lumen the area between the internal lamina elastica and the outer margins of the media was calculated by an electronic morphometric calculator (Kontron Messgerate GMBH) as a measure for the arterial medial muscle mass

D etary inquiry

A written inquiry was sent to the mother after discharge from the hospital concerning her weight 10 days after delivery iron and vitamin supplementation and food intake during pregnancy

Stat sti al methods

p-Values were obtained by means of the Chi square test two-sided t test and by simple linear regression analysis

RESULTS

Table 1 presents the clinical and laboratory data concerning the mothers the infants the placentas and the umbilical cords in the two groups Smoking mothers were of lower age than non smokers and the group contained more single women than the controls who were nearly all married The groups were equal with regard to parity of the mothers mothers weight and height before pregnancy weight gain during pregnancy and weight gain of the mother herself as calculated from her weight 10 days after birth The sex distribution of the infants was equal in both groups as was gestational age

Significantly higher serum thiocyanate levels were present in smokers compared to non smokers The thiocyanate concentration in serum fell slightly from just before delivery until the 4th day after delivery however not significantly In the 21 non pregnant non smoking controls serum thiocyanate concentration was 55±15 5 μmol/l and similar to that in the pregnant non smokers ($p>0\ 05$) The thiocyanate concentration in human milk was considerably lower than in serum and no difference existed between smokers and non smokers No correlation was present between the serum and milk concentrations of thiocyanate ($p>0\ 05$) Significantly increasing thiocyanate concentrations were found with increasing cigarette consumption (Fig 1)

A highly significant correlation existed between the thiocyanate concentration of the mother within 24 hours before delivery and the umbilical cord serum thiocyanate con

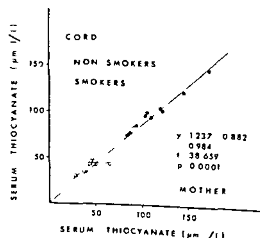


Fig 2 Relation between maternal serum thiocyanate concentrations and umbilical cord serum thiocyanate concentrations in 26 women smoking 10–20 cigarettes daily during pregnancy and in 25 non-smoking controls

Table 1 *Clinical and laboratory data on 28 smoking and 25 non smoking mothers the infants placentas and umbilical cords*

Cigarette consumption ranged 10–20 cigarettes daily during pregnancy in the smoking group *n* = number of observatio

	Smokers			Non smokers			
	n	Range	Mean ± S D	*n*	Range	Mean ± S D	*p*
Maternal age (years)	28	20–30	23 8±2 9	25	22–30	27±1 8	<0 0
Married	15	–	–	22	–	–	<0 0
Living together with the child s father	8	–	–	3	–	–	>0 0
Single	5	–	–	0	–	–	<0 0
Para 0/para 1	13/15	–	–	11/14	–	–	>0 0
Maternal height (cm)	28	158–181	166 9±5 6	25	156–178	167 5±5 5	>0 0
Maternal weight before pregnancy (kg)	25	49–83	59±9 5	23	45–85	59 3±8 7	>0 0
Total weight gain during pregnancy (kg)	24	6–21	13 2±3 9	23	8 3–24	15±4 2	>0 0
Weight gain of the mother alone (kg)	22	−2 5–16	4 4±4 5	20	−2–11	3 9±3 4	>0 0
Maternal serum thiocyanate at delivery (µmol/l)	26	52–173	109 6±29 9	25	23–82	48 2±16	<0 0
Cord serum thiocyanate (µmol/l)	26	50–149	98 2±27 2	25	24–82	43 3±13 5	<0 0
Maternal serum thiocyanate 4th day after delivery (µmol/l)	24	48–154	100 3±27 8	25	24–81	43 6±13 2	<0 0
Human milk thiocyanate 4th day after delivery (µmol/l)	24	4–53	15 6±13 2	24	3–62	18 1±15 6	>0 0
Gestational age (weeks)	28	39–42	40 4–1 1	25	39–42	40 6–1 0	>0 0
Infants sex ratio (male/female)	16/12	–	–	13/12	–	–	>0 0
Infant birth weight (g)	28	2 480–4 160	3 344±434	25	2 960–5 150	3 670±504	<0 0
Infant birth length (cm)	28	47–53 5	49 8±1 7	25	47–55	51–1 6	<0 0
Infant head circumference at birth (cm)	27	32 5–37	34 8±1 2	24	32 5–39	35 4±1 4	>0 0
Infant weight loss (g)	28	*100–290*	*173 6±50 9*	25	50–310	*170 4–68 4*	>0 0
Time for lowest weight (days after birth)	28	3–7	3 5±1 0	25	3–5	3 3±0 5	>0 0
Placental weight (with membranes) (g)	28	400–700	560±86 4	25	450–750	590±89 9	>0 0
Placental weight (without membranes) (g)	25	370–560	490±61	25	400–690	517±76	>0 05
Placental diameter (cm)	25	16 6–21	18 8±1 5	22	15 5–22 5	19 3±1 8	>0 05
Umbilical cord artery medial area (mm²)	21	1 61–3 17	2 32±0 51	17	1 47–3 44	2 42±0 59	>0 05

were the first non smoking women admitted for delivery after a mother in the smoking group and that fulfilled all the other selection criteria An additional 21 healthy non pregnant non smoking women 21–30 years old (23 4± 2 5 years mean ± S D) were investigated for serum thiocyanate concentrations

The mothers age marital status height weight before pregnancy and weight gain during pregnancy were registered from the hospital records as were weight length and head circumference of the infants at birth Infant body weight was registered daily and the loss of weight after birth calculated

Thiocyanate investigations

Venous blood samples were taken from the mothers within 24 hours before delivery and again on the 4th day after delivery Cord blood samples were taken at delivery from the placental part of the cord A milk sample was collected from the mothers on the 4th day after delivery Blood samples were rapidly centrifuged and all serum samples were stored at −30 C together with the milk samples The serum and milk samples were analyzed for thiocyanate by an automated modification of the method of Pettigrew & Fell (15)

Histological investigations

Placentas were weighed after separating the cord at the placental insertion and also weighed after stripping of the membranes fixed in 4% formalin The diameter of each placenta was registered as the mean of two angularly measured diameters The placentas were studied grossly and cut in transverse sections 1 5 cm wide

fetus of smoking mothers therefore may be exposed to toxic cyanide levels in utero

The lower concentrations of thiocyanate in human milk and the lack of correlation between serum and milk concentrations may be due to the handling of the thiocyanate ion in the mammary gland Thiocyanate shows a physical and chemical similarity to halogen ions and behaves much like chloride ions in the body (20) which also are found in low concentration in human milk compared to serum concentration

There has been discussion on whether cigarette smoking acts as an exogenous factor which interferes with intra uterine development of the fetus (1 12 13) or whether smoking women are a self selected group more likely both to smoke and to have infants with lower birth weights (10) In our study the maternal serum thiocyanate levels were inversely correlated to infant birth weight indicating that smoking itself interferes with fetal growth in a dose related manner Similar inversity has been found in far greater series between birth weight and maternal smoking levels (12 13) The growth retardation did not seem to be caused by differences in the two groups of nutritional factors during pregnancy (maternal weight gain) genetical potential for growth (maternal size) or marital status of the mothers

Ultrastructural changes are found in umbilical cord vessels after maternal smoking during pregnancy (3 4) as well as in placental vessels (2) In our study we did not investigate small changes and probably therefore were not able to show differences between smokers and non smokers

Hypertrophy of medial smooth muscle is found in conditions associated with increased vascular resistance (11) If the decreased blood flow through the placenta found in smokers (19) is caused by an increased vascular resistance it might have been reflected in hypertrophy of smooth muscle in the umbilical cord arteries We were however not able to demonstrate this with the method used

ACKNOWLEDGEMENTS

This work is supported from The Norwegian Research Council for Science and the Humanities and from The Norwegian Council on Cardiovascular Diseases

REFERENCES

1 Andrews J Thiocyanate and smoking in pregnancy *Br J Obstet Gynaecol 80* 810 1973
2 Asmussen I Ultrastructure of the human placenta at term Observations on placentas from newborn children of smoking and non smoking mothers *Acta Obstet Gynecol Scand 56* 119 1977
3 Asmussen I Ultrastructure of human umbilical vessels Observations on veins from newborn children of smoking and nonsmoking mothers *Acta Obstet Gynecol Scand 57* 253 1978
4 Asmussen I & Kjeldsen K Intimal ultrastructure of human umbilical arteries Observations on arteries from newborn children of smoking and nonsmoking mothers *Circ Res 36* 579 1975
5 Chung J & Wood J L Oxidation of thiocyanate to cyanide catalyzed by hemoglobin *J Biol Chem 246* 555 1971
6 Cole P V Hawkins L H & Roberts D Smoking during pregnancy and its effects on the fetus *Br J Obstet Gynaecol 79* 78? 1972
7 Comstock G W Shah F K Meyer M B & Abbey H Low birth weight and neonatal mortality related to maternal smoking and socioeconomic status *Am J Obstet Gynecol 111* 53 1971
8 Dunn H G McBurney A K Ingram S & Hunter C M Maternal cigarette smoking during pregnancy and the child's subsequent development I Physical growth to the age of 6½ years *Can J Public Health 67* 499 1976
9 Dunn H G McBurney A K Ingram S & Hunter C M Maternal cigarette smoking during pregnancy and the child s subsequent development II Neurological and intellectual maturation to the age of 6½ years *Can J Public Health 68* 43 1977
10 Hickey R J Clelland R C & Bowers E J Maternal smoking birth weight infant death and the self selection problem *Am J Obstet Gynecol 131* 805 1978
11 Levin D L Fixler D E Morriss F C & Tyson J Morphologic analysis of the pulmonary vascular bed in infants exposed in utero to prostaglandin synthetase inhibitors *J Pediatr 9?* 478 1978
12 MacMahon B Alpert M & Salber E J Infant weight and parental smoking habits *Am J Epidemiol 8?* ?47 1966
13 Meyer M B Jonas B S & Tonascia J A Perinatal events associated with maternal smoking during pregnancy *Am J Epidemiol 103* 464 1976
14 Murphy J F Mulchay R & Drumm J E Smoking and the fetus *Lancet II* 36 1977
15 Pettigrew A R & Fell G S Simplified colorimetric determination of thiocyanate in biological fluids and its application to investigation of the toxic amblyopias *Clin Chem 18* 996 1972

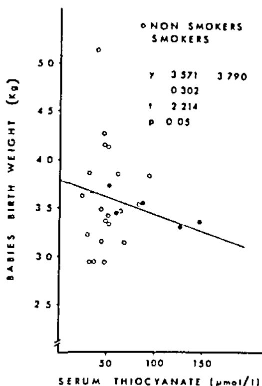


Fig. 3. Relation between maternal serum thiocyanate concentrations at delivery in 26 women smoking 10–20 cigarettes daily during pregnancy and in 25 non smoking controls and the birth weights of their infants

centration (Fig 2) and also between maternal serum thiocyanate concentration on the 4th day after delivery and the cord serum level (r=0 979 p<0 0001)

A significant inversity was found between birth weight and mother thiocyanate levels (Fig 3) There was no statistically significant correlation between the mothers estimated cigarette consumption and birth weight (r= −0 217 p>0 05)

The infants of smoking mothers had significantly lower weight and length at birth These differences were also present when calculated for only the married women in the two groups Weight loss after birth was equal in both groups of infants as was the timing for the lowest weight after birth

No difference was found on placental size (weight diameter) between smokers and non smokers Histological investigation of the placentas (25 placentas from each group) showed syncytial knots small infarctions small calcifications and fibrinoid necrosis of villi in most placentas in both groups These changes were not of any size or number suspect of any influence on placental function In sections from the umbilical cords and membranes no pathological findings were present The umbilical artery medial area was not different in smokers and non smokers

The inquiry about food intake during pregnancy showed a varied and probably adequate food intake in all mothers Twenty five smokers and 23 non smokers used iron supplements and 25 smokers and 21 non smokers a multivitamin preparation during pregnancy

DISCUSSION

Serum thiocyanate concentrations of smoking mothers were found to be positively correlated to their smoking levels (Fig 1) This is in accordance with other investigations (1 20) Pregnancy did not seem to influence the thiocyanate concentration as judged from our results in non smokers The thiocyanate concentration of serum in smokers therefore may be used as an objective measure for the smoke exposure However non smokers also have a considerable and variable amount of thiocyanate in serum mostly caused by the thiocyanate content in food (20) This limits the accuracy of the thiocyanate concentrations reflecting the smoking level

The thiocyanate concentrations in cord blood serum were found to be nearly identical with maternal serum thiocyanate concentrations at delivery as well as on the 4th day after delivery Thus cord serum thiocyanate seems to reflect the mother s smoking habits in the same way as her own serum thiocyanate concentration Whether the fetal thiocyanate level reflects only passive diffusion of thiocyanate from the mother or also cyanide detoxication in the fetus itself remains unknown However a dynamic equilibration exists between thiocyanate formed by detoxication of cyanide and reverse oxidation of thiocyanate to cyanide by enzymes present in red blood cells and other cells (5) The

SPONTANEOUS LABOUR AND ELECTIVE INDUCTION— A PROSPECTIVE RANDOMIZED STUDY

Behavioural Assessment and Neurological Examination in the Newborn Period

INGEMAR LEIJON ORVAR FINNSTROM STAFFAN HEDENSKOG
GUNNAR RYDÉN and JOHN TYLLESKAR

From the Departments of Paediatrics Obstetrics and Gynaecology University Hospital Linkoping and the Women s Clinic Central Hospital Motala Sweden

ABSTRACT Leijon I Finnstrom O Hedenskog S Ryden G and Tylleskar J (Departments of Paediatrics and Obstetrics and Gynaecology University Hospital Linkoping and the Women s Clinic Central Hospital Motala Sweden) Spontaneous Labour and elective induction—a prospective randomized study Behavioural assessment and neurological examination in the newborn period Acta Paediatr Scand 68 553 1979.—The effect of induction of labour on the foetus and the newborn was investigated in a prospective randomized study 41 neonates were studied after induction at full term by amniotomy and intravenous oxytocin infusion The infusion rate was regulated by the intraamniotic pressure using the Cardiff infusion pump system 39 neonates served as controls where the labour started spontaneously followed by amniotomy In both groups foetal heart rate monitoring and intraamniotic pressure recordings were performed There were no differences in Apgar score and pH in cord blood between the groups The newborns were evaluated the first and the fifth day of life with the Brazelton Neonatal Behavioral Assessment Scale and with a modified Prechtl neurological examination There were no differences in behaviour and neurological state between the two groups However within the groups there were significant differences between the first and the fifth day concerning both neonatal behaviour and neurological state

KEY WORDS Newborn behavioural assessment neurological examination elective induction asphyxia

The indications and methods for induction of labour have varied considerably leading to difficulties in evaluating reports of pros and cons with this procedure Induction of delivery on medical (clinical) indications i e placental insufficiency or prolonged pregnancy has been an accepted procedure for a long time Opinions differ concerning induction at full term without clinical indications

In previous retrospective studies the induction of delivery has been claimed to reduce the perinatal mortality and morbidity because of an intensified foetal monitoring and reduced frequency of postterm deliveries (12 19 21) Other authors have found an increased frequency of Dip I after induction and consider it to be a risk for developing cerebral injuries (27) Martell et al (20) have observed reduced pH in cord blood after induction with amniotomy However Alderman (1) found no difference in the frequency of low Apgar scores between spontaneous and induced labour using either low dose or titrated oxytocin techniques Follow up studies of children born after elective induction have not demonstrated any negative effects compared with control groups (18 22) There are only a few reports in which the effect of induced delivery has been studied prospectively No studies have been published in which the neurological and behavioural state of the newborn infants have been examined after induced delivery In the

16 Pettigrew A R Logan R W & Willcocks J Smoking in pregnancy—effects on birth weight and on cyanide and thiocyanate levels in mother and baby *Br J Obstet Gynaecol 84* 31 1977
17 Prigge E & Hochrainer D Effects of carbon monoxide inhalation on erythropoiesis and cardiac hypertrophy in fetal rats *Toxicol Appl Pharmacol 42* 225 1977
18 Rush D & Kass E H Maternal smoking A reassessment of the association with perinatal mortality *Am J Epidemiol* 96 183 1972
19 Spira A Fournier R Grob J C Dreyfus J & Schwartz D Smoking during pregnancy and placental blood flow *Proc 6th Congress of Perinatal Medicine* Vienna 1978
20 Stoa K F *Studies on thiocyanate in serum* 2nd Medical Yearbook University of Bergen 1957

Submitted Nov 21 1978
Accepted Febr 7 1979

(A M) Department of Paediatrics
Ullevål Hospital
Oslo
Norway

Acta Pædiatr Scand 68 553–560 1979

SPONTANEOUS LABOUR AND ELECTIVE INDUCTION— A PROSPECTIVE RANDOMIZED STUDY

Behavioural Assessment and Neurological Examination in the Newborn Period

INGEMAR LEIJON ORVAR FINNSTRÖM STAFFAN HEDENSKOG
GUNNAR RYDEN and JOHN TYLLESKAR

From the Departments of Paediatrics Obstetrics and Gynaecology University Hospital Linkoping and the Women s Clinic Central Hospital Motala Sweden

ABSTRACT Leijon I Finnstrom O Hedenskog S Ryden G and Tylleskar J (Departments of Paediatrics and Obstetrics and Gynaecology University Hospital Linkoping and the Women s Clinic Central Hospital Motala Sweden) Spontaneous Labour and elective induction—a prospective randomized study Behavioural assessment and neurological examination in the newborn period Acta Paediatr Scand 68 553 1979 —The effect of induction of labour on the foetus and the newborn was investigated in a prospective randomized study 41 neonates were studied after induction at full term by amniotomy and intravenous oxytocin infusion The infusion rate was regulated by the intraamniotic pressure using the Cardiff infusion pump system 39 neonates served as controls where the labour started spontaneously followed by amniotomy In both groups foetal heart rate monitoring and intraamniotic pressure recordings were performed There were no differences in Apgar score and pH in cord blood between the groups The newborns were evaluated the first and the fifth day of life with the Brazelton Neonatal Behavioral Assessment Scale and with a modified Prechtl neurological examination There were no differences in behaviour and neurological state between the two groups However within the groups there were significant differences between the first and the fifth day concerning both neonatal behaviour and neurological state

KEY WORDS Newborn behavioural assessment neurological examination elective induction asphyxia

The indications and methods for induction of labour have varied considerably leading to difficulties in evaluating reports of pros and cons with this procedure Induction of delivery on medical (clinical) indications i e placental insufficiency or prolonged pregnancy has been an accepted procedure for a long time Opinions differ concerning induction at full term without clinical indications

In previous retrospective studies the induction of delivery has been claimed to reduce the perinatal mortality and morbidity because of an intensified foetal monitoring and reduced frequency of postterm deliveries (12 19 21) Other authors have found an increased frequency of Dip I after induction and consider it to be a risk for developing cerebral injuries (27) Martell et al (20) have observed reduced pH in cord blood after induction with amniotomy However Alderman (1) found no difference in the frequency of low Apgar scores between spontaneous and induced labour using either low dose or titrated oxytocin techniques Follow up studies of children born after elective induction have not demonstrated any negative effects compared with control groups (18 22) There are only a few reports in which the effect of induced delivery has been studied prospectively No studies have been published in which the neurological and behavioural state of the newborn infants have been examined after induced delivery In the

16 Pettigrew A R Logan R W & Willcocks J Smoking in pregnancy—effects on birth weight and on cyanide and thiocyanate levels in mother and baby *Br J Obstet Gynaecol 84* 31 1977
17 Prigge E & Hochrainer D Effects of carbon monoxide inhalation on erythropoiesis and cardiac hypertrophy in fetal rats *Toxicol Appl Pharmacol 42* 225 1977
18 Rush D & Kass E H Maternal smoking A reassessment of the association with perinatal mortality *Am J Epidemiol* 96 183 1972
19 Spira A Fournier R Grob J C Dreyfus J & Schwartz D Smoking during pregnancy and placental blood flow *Proc 6th Congress of Perinatal Medicine* Vienna 1978
20 Stoa K F *Studies on thiocyanate in serum* and Medical Yearbook University of Bergen 1957

Submitted Nov 21 1978
Accepted Febr 7 1979

(A M) Department of Paediatrics
Ullevål Hospital
Oslo
Norway

Table 2 *Behavioural items in the Brazelton Neonatal Assessment Scale*

Lability of skin colour and smiles are excluded

Dimension 1 Interactive processes	Orientation inanimate visual (red ball); Orientation inanimate auditory (rattle); Orientation animate visual (face); Orientation animate auditory (voice); Orientation animate visual and auditory (face and voice); Alertness; Cuddliness; Consolability
Dimension 2 Motor processes	General tonus; Pull to sit; Motor maturity; Defensive movements; Hand to mouth activity; Activity level
Dimension 3 Organizational processes control of state	Response decrement to light; Response decrement to rattle; Response decrement to bell; Response decrement to pinprick; Peak of excitement; Rapidity of buildup; Lability of states; Irritability; Self-quieting activity
Dimension 4 Organizational processes response to stress	Tremulousness; Startles

chloride 50 mg (pethidine—Atarax®) by *intramuscular* injection and pudendal block with 20 ml 0 5% Mepivacaine chloride (Carbocain®)

Procedure at delivery

Immediately after delivery the newborn infant was placed on the delivery bed at the level of the vaginal outlet Exactly 60 se after delivery the cord was clamped and a blood sample from the umbilical vein was drawn for pH analysis 0 2 mg methyl ergometrine (Methergin®) was then given to the mother

Examination of the infant

The infants were evaluated according to Apgar at 1 and 5 min During the second day a maturity assessment using external characteristics was performed (14) The infants were examined on days 1 and 5 with the Brazelton Neonatal Behavioural Assessment scale (9) These examinations were done without knowledge of which group the infants belonged to and in a calm surrounding roughly hours after feeding Standard administration procedures were followed but two items lability of skin colour and smiles were not analyzed All infants were examined by the same examiner (I L) who had been trained to reliability by the Brazelton group The Brazelton Examination

Table 3 *Grouping of items in the neurological examination*

Figures in parenthesis are possible scores

Items measuring muscular tonus (8–31)
Spontaneous posture supine position (1–3)
Resistance against passive movements body and neck (1–4)
Resistance against passive movements arms (1–4)
Resistance against passive movements legs (1–4)
Recoil arms (1–4)
Traction response arms (1–4)
Traction response head (1–4)
Head control vertical position (1–4)

Items measuring excitability (9–33)
Optical blink reflex (1–3)
Acoustic blink reflex (1–3)
Patellar reflex (1–4)
Palmar grasp (1–4)
Withdrawal reflex (1–4)
Rooting reflex (1–4)
Ability to suck (1–4)
Moro tremor (1–3)
Spontaneous motility (1–4)

Optimal items (Items where optimal response is possible to state)
The items measuring muscular tonus (see above)
The items measuring excitability (see above Moro tremor excluded)
Face expression (1–?)
Cry (1–4)
Symmetry in body position (1–2)
Eyes following a red ball (1–3)
Dolls eye test (1–)
Pupil s reaction to light (1–3)
Glabellar reflex (1–?)
Moro reflex (1–4)
Crawling (1–4)
Placing response (1–2)
Automatic walking (1–3)

Total neurological score (36–126)
Optimal items (see above) (?7–9?)
Neurological symptoms
Drowsiness (1–3)
Jitteriness (1–?)
Convulsions (1–?)
Others (1–?)
Fontanel tension (1–6)
Position and movements of eyes (1–?)
Side difference in resistance against passive movements (1–?)
Asymmetric Moro (1–2)
Moro tremor (1–3)

assesses the infant s behaviour with regard to 27 items in a nine point scale see Table ? The items were organized in four dimensions according to Als et al (? 3) 1 Interactive processes i e the infant s capacity for orientation to external stimuli and to respond to social stimuli ? Motor processes i e the infant s ability to control motor behaviour and muscle tonus 3 Organization processes

Table 1 *The number and the age of the women in induced and spontaneous labour*
Mean and S D

	Induced labour		Spontaneous labour	
	n	Mean age	*n*	Mean age
Primiparae	18	22 4±4 5	18	23 6±3 4
Multiparae	23	26 3±4 0	21	25 0±3 1

present prospective randomized study infants have been compared after elective induction and spontaneous delivery at fullterm The risk for asphyxia has been studied as well as the behavioural and neurological state of the new born infants

MATERIAL AND METHODS

Selection of patients

The investigation was performed at the Departments of Paediatrics and Obstetrics at the University Hospital Linköping and the Central Hospital Motala Sweden Mothers were selected at the regular prenatal visits during the later part of pregnancy Primiparae as well as multiparae fulfilling the following criteria were included

1 Maternal age between 18 and 30 years for primiparae and 18 to 35 for multiparae

2 The last menstrual period normal and known Regular menstrual periods before the actual pregnancy Women using hormonal contraceptives should have had at least three normal periods after completed medication

3 Previous pregnancies and deliveries normal with birth weights between 3 000 and 4 000 grams

4 Normal symphysis–fundus distance and weight gain according to gravidogram (32)

5 The present pregnancy normal and the foetus in vertex presentation

6 Normal pelvic outlet according to clinical examination

Patients fulfilling these criteria were examined within one week before expected date of delivery The condition of the cervix was estimated in a ten point scale modified after Bishop (5 31) If a pelvic score of at least 5 points for primiparae and at least 4 points for multiparae was found the patient was invited to participate in the study The date of planned delivery was decided to ±2 days from the date of expected delivery If the woman agreed to participate she was randomly put into one of two groups In group I deliveries were induced and in group II deliveries were allowed to start spontaneously Patients in whom the labour started spontaneously earlier than the date of planned delivery were excluded from the investigation If the pregnancy in group II was prolonged more than 14 days from the date of estimated delivery the delivery was induced according to the routine clinical indications in the departments These patients belonged to the original group

112 women fulfilling the criteria given above agreed to participate mean age 24 5±4 0 years 13 in the induction group (group I) and 12 in the spontaneous group (group II) were delivered before the date of planned delivery 3 mothers in group II had prolonged pregnancies As the number of these patients were few and did not affect the results they were included in the study There were 2 Caesarean sections due to disproportion between the foetal head and the maternal pelvis These two infants were in good condition but were excluded from the investigation The first five infants in the study were not completely examined and were excluded A total of 80 infants were examined 41 in group I and 39 in group II The distribution and the age of the women in the groups are shown in Table 1 Seventeen of the infants were born and examined in Linköping the others in Motala

Induction of delivery—group I

On the morning of the day of planned delivery amniotomy was performed through an amnioscope and a catheter for registration of intraamniotic pressure was inserted A scalp electrode was applied on the foetal head for registration of foetal heart frequency The registrations were performed using the Hewlett Packard or Corometrics equipment

Oxytocin was infused intravenously using the Cardiff infusion pump system Mark II (11 15) An alarm for intrauterine pressure higher than 80 mmHg and for uterine spasm (intraamniotic pressure >35 mmHg for more than 120 sec) was attached to the system The infusion was started 15 min after amniotomy with an initial infusion rate of 1 mμ/min and was increased continuously until the intensity of the contractions reached at least 35 mmHg and a frequency of at least one contraction every 150 sec The infusion was stopped immediately after birth

Spontaneous delivery—group II

The women were asked to come to the delivery wards as soon as labour started External CTG registration was performed until an established labour could be demonstrated This was thought to be the situation when the frequency of contractions was at least one every 5 min the cervix was effaced at least 50% and dilated at least 2 cm for primiparae and at least 3 cm for multiparae Amniotomy was now done a catheter for intrauterine registration of pressure and a scalp electrode was applied The registrations were then performed in the same manner as in group I

The progress of labour was followed every hour with regard to the degree of dilatation of the cervix and the position of the head in the pelvis and was registered in a partogram

Obstetric analgesia

To reduce as far as possible the influence of obstetric analgesia on the labour and the foetus only a limited number of methods were allowed Nitrous oxide-oxygen 50% of each pethidine chloride 50 mg with hydroxizine

Table 2 *Behavioural items in the Bra elton Neonatal Assessment Scale*

Lability of skin colour and smiles are excluded

Dimension 1 Interactive proce ses	Orientation inanimate visual (red ball) Orientation inanimate auditory (rattle) Orientation animate visual (face) Orientation animate auditory (voice) Orientation animate visual and auditory (face and voice) Alertness Cuddliness Consolability
Dimension 2 Motor processes	General tonus Pull to sit Motor maturity Defensive movements Hand to mouth activity Activity level
Dimension 3 Organizational processes control of state	Response decrement to light Response decrement to rattle Response decrement to bell Response decrement to pinprick Peak of excitement Rapidity of buildup Lability of states Irritability Self-quieting activity
Dimension 4 Organizational processes response to stress	Tremulousness Startles

Table 3 *Grouping of items in the neurological examination*

Figures in parenthesis are possible scores

Items measuring muscular tonus (8–31)
- Spontaneous po ture supine position (1–3)
- Resistance against passive movements body and neck (1–4)
- Resistance against passive movements arms (1–4)
- Resistance against passive movements legs (1–4)
- Recoil arms (1–4)
- Traction response arms (1–4)
- Traction response head (1–4)
- Head control vertical position (1–4)

Items measuring excitability (9–33)
- Optical blink reflex (1–3)
- Acoustic blink reflex (1–3)
- Patellar reflex (1–4)
- Palmar grasp (1–4)
- Withdrawal reflex (1–4)
- Rooting reflex (1–4)
- Ability to suck (1–4)
- Moro tremor (1–3)
- Spontaneous motility (1–4)

Optimal items (Items where optimal response is possible to state)
- The items measuring muscular tonus (see above)
- The items measuring excitability (see above Moro tremor excluded)
- Face expression (1–2)
- Cry (1–4)
- Symmetry in body position (1–)
- Eyes following a red ball (1–3)
- Dolls eye test (1–)
- Pupil s reaction to light (1–3)
- Glabellar reflex (1–2)
- Moro reflex (1–4)
- Crawling (1–4)
- Placing response (1–2)
- Automatic walking (1–3)

Total neurological score (36–1 6)
- Optimal items (see above) (27–9)
- Neurological symptoms
  - Drowsines (1–3)
  - Jitterness (1–)
  - Convulsions (1–2)
  - Others (1–2)
- Fontanel tension (1–6)
- Position and movements of eyes (1–2)
- Side difference in resistance against pa sive movements (1–2)
- Asymmetric Moro (1–2)
- Moro tremor (1–3)

chloride 50 mg (pethidine—Atarax®) by intramuscular injection and pudendal block with 20 ml 0 5% Mepivacaine chloride (Carbocain®)

P ocedure at del ery

Immediately after delivery the newborn infant was placed on the delivery bed at the level of the vaginal outlet Exactly 60 sec after delivery the cord was clamped and a blood sample from the umbili al vein was drawn for pH analysis 0 mg methyl ergometrine (Methergin®) was then given to the mother

Examination of the infant

The infants were evaluated according to Apgar at 1 and 5 min During the second day a maturity assessment using external characteristics was performed (14) The infants were examined on days 1 and 5 with the Brazelton Neonatal Behavioural Asses ment scale (9) These examinations were done without knowledge of which group the infants belong d to and in a calm surrounding roughly 2 hours after feeding Standard administration procedures were followed but two i ems lability of skin colour and smiles were not analyzed All infants were examined by the same examiner (I L) who had been trained to reliability by the Brazelton group The Brazelton Examination assesses the infant s behaviour with regard to 27 items in a nine point scale see Table 2 The items were organized in four dimensions according to Als et al (? 3) 1 Interactive processes i e the infant s capacity for orientation to external stimuli and to respond to social stimuli 2 Motor processes i e the infant s ability to control motor behaviour and muscle tonus 3 Organization processes

Table 1 *The number and the age of the women in induced and spontaneous labour*
Mean and S D

	Induced labour		Spontaneous labour	
	n	Mean age	*n*	Mean age
Primiparae	18	22 4±4 5	18	23 6±3 4
Multiparae	23	26 3±4 0	21	25 0±3 1

present prospective randomized study infants have been compared after elective induction and spontaneous delivery at fullterm The risk for asphyxia has been studied as well as the behavioural and neurological state of the new born infants

MATERIAL AND METHODS

Selection of patients

The investigation was performed at the Departments of Paediatrics and Obstetrics at the University Hospital Linkoping and the Central Hospital Motala Sweden Mothers were selected at the regular prenatal visits during the later part of pregnancy Primiparae as well as multiparae fulfilling the following criteria were included

1 Maternal age between 18 and 30 years for primiparae and 18 to 35 for multiparae

2 The last menstrual period normal and known Regular menstrual periods before the actual pregnancy Women using hormonal contraceptives should have had at least three normal periods after completed medication

3 Previous pregnancies and deliveries normal with birth weights between 3 000 and 4 000 grams

4 Normal symphysis-fundus distance and weight gain according to gravidogram (37)

5 The present pregnancy normal and the foetus in vertex presentation

6 Normal pelvic outlet according to clinical examination

Patients fulfilling these criteria were examined within one week before expected date of delivery The condition of the cervix was estimated in a ten point scale modified after Bishop (5 31) If a pelvic score of at least 5 points for primiparae and at least 4 points for multiparae was found the patient was invited to participate in the study The date of planned delivery was decided to ±2 days from the date of expected delivery If the woman agreed to participate she was randomly put into one of two groups In group I deliveries were induced and in group II deliveries were allowed to start spontaneously Patients in whom the labour started spontaneously earlier than the date of planned delivery were excluded from the investigation If the pregnancy in group II was prolonged more than 14 days from the date of estimated delivery the delivery was induced according to the routine clinical indications in the departments These patients belonged to the original group

112 women fulfilling the criteria given above agreed to participate mean age 24 5±4 0 years 13 in the induction group (group I) and 12 in the spontaneous group (group II) were delivered before the date of planned delivery 3 mothers in group II had prolonged pregnancies As the number of these patients were few and did not affect the results they were included in the study There were 2 Caesarean sections due to disproportion between the foetal head and the maternal pelvis These two infants were in good condition but were excluded from the investigation The first five infants in the study were not completely examined and were excluded A total of 80 infants were examined 41 in group I and 39 in group II The distribution and the age of the women in the groups are shown in Table 1 Seventeen of the infants were born and examined in Linkoping the others in Motala

Induction of delivery—group I

On the morning of the day of planned delivery amniotomy was performed through an amnioscope and a catheter for registration of intraamniotic pressure was inserted A scalp electrode was applied on the foetal head for registration of foetal heart frequency The registrations were performed using the Hewlett Packard or Corometrics equipment

Oxytocin was infused intravenously using the Cardiff infusion pump system Mark II (11 15) An alarm for intra uterine pressure higher than 80 mmHg and for uterine spasm (intraamniotic pressure >35 mmHg for more than 120 sec) was attached to the system The infusion was started 15 min after amniotomy with an initial infusion rate of 1 mμ/min and was increased continuously until the intensity of the contractions reached at least 35 mmH and a frequency of at least one contraction every 150 sec The infusion was stopped immediately after birth

Spontaneous delivery—group II

The women were asked to come to the delivery wards as soon as labour started External CTG registration was performed until an established labour could be demonstrated This was thought to be the situation when the frequency of contractions was at least one every 5 min the cervix was effaced at least 50% and dilated at least 2 cm for primiparae and at least 3 cm for multiparae Amniotomy was now done a catheter for intrauterine registration of pressure and a scalp electrode was applied The registrations were then performed in the same manner as in group I

The progress of labour was followed every hour with regard to the degree of dilatation of the cervix and the position of the head in the pelvis and was registered in a partogram

Obstetric analgesia

To reduce as far as possible the influence of obstetric analgesia on the labour and the foetus only a limited number of methods were allowed Nitrous oxide-oxygen 50% of each pethidine chloride 50 mg with hydroxizine

Table 5 *The four dimensions in the Brazelton Scale (Brazelton Neonatal Assessment Scale 1973) in neonates born after induced and spontaneous labour*

The table shows the results on day 1 and 5 of the number and percentage of neonates in each performance score mean and standard deviation (1 S D) of performance score Performance score 1 means very good performance average performance and 3 poor performance

Performance score	Day 1				Day 5			
	Induced labour		Spontaneous labour		Induced labour		Spontaneous labour	
	n	%	*n*	%	*n*	%	*n*	%
Dimension 1	*n*=41		*n*=36		*n*=41		*n*=38	
1	6	14 6	4	11 1	10	[illegible]4 4	7	18 4
[illegible]	31	75 6	[illegible]4	66 7	[illegible]9	70 7	[illegible]9	76 3
3	4	9 8	8	[illegible]	[illegible]	4 9	2	5 3
Mean	1 95±0 50		[illegible] 11±0 58		1 80±0 51		1 87±0 48	
Dimension [illegible]	*n*=41		*n*=35		*n*=41		*n*=38	
1	0	0	[illegible]	5 7	2	4 9	4	10 5
	30	73	[illegible]1	60 0	39	95 1	34	89 5
3	11	6 8	1	34 3	0	0	0	0
Mean	[illegible] 7±0 45		[illegible] 9±0 57		1 95±0 [illegible]		1 89±0 31	
Dimension 3	*n*= 8		*n*=[illegible]5		*n*= 6		*n*=[illegible]	
1	5	17 9	7	8 0	3	11 5	3	13 6
[illegible]		78 6	18	7[illegible] 0	18	69 [illegible]	16	7 7
3	1	3 6	0	0	5	19	3	13 6
Mean	1 86±0 45		1 7[illegible]±0 46		[illegible] 08±0 56		2 00±0 54	
Dimension 4	*n*=41		*n*=36		*n*=41		*n*=38	
1	30	73 [illegible]	9	80 6	40	97 6	36	94 7
3	11	[illegible]6 8	7	19 4	1	[illegible] 4	2	5 3
Mean	1 54±0 90		1 39±0 80		1 05±0 31		1 11±0 45	

dimension 2 $t=6\ 11$ $p<0\ 001$ dimension 3 $t=2\ 23$ $p<0\ 05$ dimension 4 $t=4\ 14$ $p<0\ 001$) The differences between day 1 and 5 indicate that on day 5 more infants showed a better orientation to external stimuli and a higher alertness (dimension 1) more infants had a better ability to control motor behaviour (dimension 2) more infants had less tremulousness and startles (dimension 4) but fewer infants had a good state control (dimension 3)

Table 6 *Mean and standard deviation for the sum of four different groupings in the neurological examination*

A high value in tonus score means a low muscular tonus A high value in excitability score means low degree of excitability

	Day 1		Day 5	
	Induced labour (*n*=41)	Spontaneous labour (*n*=39)	Induced labour (*n*=41)	Spontaneous labour (*n*=39)
Tonus score (optimal=14) range 8–30	14 05±0 70	13 86±0 59	14 1 ±0 55	14 [illegible] ±0 90
Excitability score (optimal=14) range 9–33	14 83±1 9	14 83±1 54	14 0 ±0 54	13 97±0 70
Total number of optimal item (max [illegible]7)	[illegible]5 41±1 59	[illegible]4 89±1 85	[illegible]6 3[illegible]±1 0	[illegible]6 14±1 50
[illegible]tal score range 37–116	5[illegible] 93± 30	53 [illegible]± 81	51 56±1 40	51 61±1 61

Table 4. *Birth weight, lowest weight during the first week, gestational age, gestational age according to maturity assessment, Apgar scores and pH in umbilical vein in induced and spontaneous labour*

Mean ± S.D.

	Induced labour ($n=41$)	Spontaneous labour ($n=39$)
Birth weight, grams	3638±453	3720±499
Lowest weight during the first week, grams	3376±436	3448±458
Gestational age, days	280±1	285±4
Gestational age according to maturity assessment, days	280±12	281±8
Apgar score 1 min	8.8±0.7	9.0±0.4
Apgar score 5 min	9.9±0.4	9.9±0.3
pH, umbilical vein	7.35±0.07	7.33±0.07

i.e. the infant's ability to control and organize his state. 4. Physiological stability in response to stress. For each infant a performance score was calculated ranging from 1 to 3 points for each dimension (3), whereby 1 means a very good performance, 2 a fair performance and 3 a poor performance.

A neurological examination based mainly on Prechtl & Beintema (23) was carried out on day 1 and 5 at the same occasion as the behavioural assessment. The examination included 37 items, most of them evaluated in a four degree scale. Some of these items were grouped in those evaluating mainly muscle tonus or excitability (Table 3). A tonus score and an excitability score were calculated according to Schulte et al. (26). The number of optimal responses and a total neurological score were also calculated for each infant.

All infants were breast fed freely and a naked weight was recorded daily.

Statistical methods

Conventional statistical methods were employed. Student's *t* test was used throughout.

The investigation was approved by the Ethical Committee of the University of Linköping.

RESULTS

The results for the two clinics have been pooled, as there were no differences between them. The statistical analyses were made with regard to parity, but as there were no differences in these respects primiparae and multiparae have been combined in the further presentation of the results.

One infant to a primipara was born in the occiput posterior position, all the others in the occiput anterior position. There were two vacuum extractions in the spontaneous group, one because of prenatal asphyxia and one because of uterine inertia. There was a third vacuum extraction in the induction group due to prenatal asphyxia. One infant in the induction group was evaluated as preterm at the external maturity assessment. There were no differences between the groups in the use of analgesia in primiparae. However 87% of multiparae in group I and 43% of multiparae in group II were given pethidine chloride-hydroxizine chloride during labour ($p<0.01$).

The mean values for gestational age and maturity assessment did not differ between the two groups. There were no statistically significant differences in birth weight, weight reduction, Apgar scores at 1 and 5 min or in cord pH (see Table 4).

In the behavioural assessment, mean values for each item were compared for the two groups. The two groups were also compared with regard to a performance score within the four dimensions. There was a considerable drop out within dimension three which includes items measuring the infant's ability to control and organize his state (17). 53 infants could be evaluated on day 1 and 48 on day 5. The reason for drop out was that items measuring the response decrement to external stimuli could not be evaluated because the infant changed his state of arousal during this period of the examination. The drop out for the other items was small.

There were no statistically significant differences between the two groups regarding the individual items. The results of the four dimensions are described in Table 5. The distribution and percentage of infants in performance score 1–3 for the four dimensions did not show any significant differences between the groups. Comparison of the distribution of infants of each performance score on days 1 and 5 gave significant differences for all the four dimensions (dimension 1, $t=2.72$, $p<0.01$,

Table 5 *The four dimensions in the Bra.lton Scale (Bra.lton Neonatal Assessment Scale 1973) in neonates born after induced and spontaneous labour*

The table shows the results on day 1 and 5 of the number and percentage of neonates in each performance score mean and standard de iation (1 S D) of performance score Performance score 1 means very good performance 2 average performance and 3 poor performan e

Performance score	Day 1				Day 5			
	Induced labour		Spontaneous labour		Induced labour		Spontaneous labour	
	n	%	n	%	n	%	n	%
Dimen ion 1	$n=41$		$n=36$		$n=41$		$n=38$	
1	6	14 6	4	11 1	10	4 4	7	18 4
2	31	75 6	4	66 7	9	70 7	29	76 3
3	4	9 8	8	2		4 9	2	5 3
Mean	1 95±0 50		2 11±0 58		1 80±0 51		1 87±0 48	
Dimension	$n=41$		$n=35$		$n=41$		$n=38$	
1	0	0	2	5 7	2	4 9	4	10 5
2	30	73	21	60 0	39	95 1	34	89 5
3	11	6 8	1	34 3	0	0	0	0
Mean	7±0 45		29±0 57		1 95±0		1 89±0 31	
Dimension 3	$n=8$		$n=5$		$n=6$		$n=$	
1	5	17 9	7	8 0	3	11 5	3	13 6
		78 6	18	7 0	18	69 2	16	7
3	1	3 6	0	0	5	19 2	3	13 6
Mean	1 86±0 45		1 72±0 46		2 08±0 56		2 00±0 54	
Dimension 4	$n=41$		$n=36$		$n=41$		$n=38$	
1	30	73	9	80 6	40	97 6	36	94 7
3	11	6 8	7	19 4	1	- 4		5 3
Mean	1 54±0 90		1 39±0 80		1 05±0 31		1 11±0 45	

dimension 2 $t=6\ 11$ $p<0\ 001$ dimension 3 $t=2\ 23$ $p<0\ 05$ dimension 4 $t=4\ 14$ $p<0\ 001$) The differences between day 1 and 5 indicate that on day 5 more infants showed a better orientation to external stimuli and a higher alertness (dimension 1) more infants had a better ability to control motor behaviour (dimension 2) more infants had less tremulousness and startles (dimension 4) but fewer infants had a good state control (dimension 3)

Table 6 *Mean and standard deviation for the sum of four different groupings in the neurological examination*

A high value in tonus score means a low muscular tonus A high value in excitability score means low degree of excitability

	Day 1		Day 5	
	Induced labour ($n=41$)	Spontaneou labour ($n=39$)	Induced labour ($n=41$)	Spontaneous labour ($n=39$)
Tonus s ore (optimal=14) range 8–30	14 05±0 70	13 86+0 59	14 12±0 55	14 2 ±0 90
Excitability score (optimal=14) range 9–33	14 83±1 9	14 83±1 54	14 0 ±0 54	13 97±0 70
Total number of optimal item (max)	5 41±1 59	4 89±1 85	6 3 ±1 0	6 14±1 50
Total score range 3 -116	5 93± 30	53 2 ± 81	51 56±1 40	51 61±1 61

There were no differences between the two groups in this respect

The neurological investigation gave no significant differences between the two groups for the tonus score the excitability score the total number of optimal responses or the total score see Table 6 Comparing the results on day 1 and 5 there was no difference in tonus score (t=1 86 n s) There was however a difference in the excitability score (t=4 81 p<0 001) showing that the infants on day 1 were less excitable On the first day the infants had fewer optimal points (t=6 09 p<0 001) and a higher total score (t=5 98 p<0 001) There were no differences between the two groups induced or spontaneously delivered in this respect

DISCUSSION

In this investigation other facts than the induction procedure which could possibly influence the infant s neurological status have as far as possible been kept constant Factors such as the gestational age (25) and analgesia (13) influence the neurological function postnatally Through the selection procedure of the mothers the gestational ages were well known and all infants were probably born at term This is important since an unexpected preterm delivery after induction might lead to an increased risk for the infant (6 7 16) Although the obstetrical analgesia was restricted there was a difference in multiparae in the use of pethidine chloride–hydroxizine chloride This fact did not effect the results as there were no differences between the groups

The time of amniotomy has also been standardized in the spontaneous group Because of the early amniotomy the spontaneous group is comparable with the induced group in this respect The amniotic pressure was recorded in all deliveries This procedure probably reduces the risk for iatrogenic hypertonic labour and thus the risk for foetal asphyxia The foetal heart rate was recorded continuously during delivery There were no differences in foetal heart rate changes Apgar score and cord pH between the two groups These results have been discussed in detail elsewhere (31)

In order to demonstrate a possible negative influence on the neurological function of the newborn infant we used a well standardized neurological examination technique based mainly upon Prechtl & Beintema (23) This method has been used in several neonatal studies (24) in which early neurological findings could be correlated to risk pregnancies and risk deliveries The results of the present study did not show any significant differences between the groups We examined the infants on two occasions day 1 in order to better evaluate the effects of the delivery per se and day 5 when the possible influences of the delivery should be minimal (4 23) We actually found differences between day 1 and 5 showing that the infants on day 1 as a rule were less excitable These results are in agreement with Beintema (4) The infants on day 1 also had a lower optimal score and a higher total neurological score indicating the normal developmental course during the first days of life described by Beintema (4) The two groups did not differ however with regard to changes from day 1 to 5

According to some authors (3 28) a neurological examination technique is not sensitive enough to show minor dysfunction of the central nervous system of the newborn A behavioural assessment is claimed to detect such minor dysfunctions better The Brazelton Scale has been used both clinically and as a research instrument in investigations of obstetrical anesthesia and its effects on newborn behaviour (8 30) on narcotic addicted newborns (28 29) and on nutritionally deprived newborn infants (10) This assessment which is rather time consuming is said to evaluate the infants ability to react to different stimuli and to evaluate the infants ability to control his state The reproducibility of the Brazelton s method is not very well documented and factors such as the author s experience and the

circumstances surrounding the examination might influence the results In the present examination we tried to reduce this variation and only one trained person performed the behavioural assessment and the neurological examination of all infants The behavioural assessment was standardized with respect to the surroundings and the time of day when the examinations were performed Our own reproducibility was equivalent to Brazelton s (9) Further views on the method and its reproducibility will be published separately As mentioned earlier there was a considerable drop out in dimension 3 However this drop out did not differ between the two groups

There were no significant differences between the two groups within any of the four dimensions However the examinations showed that the number of infants with good performance increased from day 1 to 5 within dimensions 1 2 and 4 In dimension 3 where most items reflect the infants temperament and excitability (arousal) (17) the results showed a more normal distribution of infants in each performance score on day 5 compared to day 1 This agrees with the results of the neurological investigation in which the infants in general were less excitable on day 1 as mentioned above

The present investigation thus showed that induction of labour at full term after a normal pregnancy a normal foetal growth and a ripe cervix had no negative effects on the infant s behavioural and neurological state Other prerequisites for this result were known gestational age induction with oxytocin guided by intrauterine pressure registration and a continuous foetal heart rate recording throughout the delivery

REFERENCES

1 Alderman B Dangers of oxytocin—induced labour to fetuses *Br Med J IV* 44 19 4

2 Als H Tronick E Adamson L & Brazelton T B The behavior of the full term but underweight newborn infant *Dev Med Child Neurol 18* 590 1976

3 Al H Tronick E Lester B & Brazelton T B The Brazelton Neonatal Behavioral Assessment Scale (BNBAS) *J Abnorm Psych* 5 215 1977

4 Beintema D J A neurological study of newborn infants Clinics in *Developmental Medicine* No 28 Heinemann London 1968

5 Bishop E Pelvic scoring for elective induction *Obstet Gynecol* 24 266 1964

6 Blacow M Smith M N Graham M & Wilson R G Induction of labour *Lancet I* 217 1975

7 Booth J H & Kurdjak V B Elective induction of labour a controlled study *Can Med Assoc J 103* 45 1970

8 Brackbill Y Kane J Manniello R L & Abramson D Obstetric premedication and infant outcome *Am J Obstet Gynecol 118* 377 1974

9 Brazelton T B Neonatal Behavioral Assessment Scale *Clinics in Developmental Medicine* No 50 Heinemann London 1973

10 Brazelton T B Tronick E Lechtig A Lasky R E & Klein R E The behavior of nutritionally deprived Guatemalan infants *Dev Med Child Neurol 19* 364 1977

11 Butterworth M J Automated incremental oxytocin infusion in the induction of labor *Obstet Gynecol* 44 238 1974

12 Cole R A Howie P W & Macnaughton M C Elective induction of labour A randomised prospective trial *Lancet I* 767 1975

13 Dubowitz V Neurological fragility in the newborn Influence of medication in labour *Br J Anaesth* 47 1005 1975

14 Finnstrom O Studies on maturity in newborn infants II External characteristics *Acta Paediatr Scand 61* 24 1972

15 Francis J G Turnbull A C & Thomas F F Automatic oxytocin infusion equipment for induction of labour *J Obstet Gynaecol Br Commonw 77* 594 1970

16 Keettel W C Randall J H & Donelly M M The hazards of elective induction of labor *Am J Obstet Gynecol* 75 496 1958

17 Lester M B Emory E K & Hoffman S L A multivariate study of the effects of high risk factors on performance on the Brazelton Neonatal Assessment Scale *Child Dev* 47 515 1976

18 McBride W C Lyle I G Black B Brown C & Thomas D A study of five year old children born after elective induction of labour *Med J Aust* 2 456 1977

19 McNay M B McIlwaine G M Howie P W & Macnaughton M C Perinatal deaths analysis by clinical cause to assess value of induction of labour *Br Med J I* 34 1977

20 Martell M Belizan J M Nieto F & Schwarcz R Blood acid–base balance at birth in neonates from labors with early and late rupture of membranes *J Pediatr* 89 963 1976

21 Martius G Terminierung der Entbindung und perinatale Sterblichkeit *Dtsch Med Wochenschr 101* 489 1976

22 Niswander K R Turoff B B & Romans J

Developmental status of children delivered through elective induction of labor Obstet Gynecol 27 15 1966
23 Prechtl H F R & Beintema D The neurological examination of the fullterm newborn infant Clinics in Developmental Medicine No 12 Heinemann London 1964
24 Prechtl H F R Neurological sequelae of prenatal and perinatal complications Br Med J IV 763 1967
25 Saint Anne Dargassies S La maturation neurologique du prématuré Etud Neo natal 4 71 1955
26 Schulte F J Schrempf G & Hinze G Maternal toxemia fetal malnutrition and motor behavior of the newborn Pediatrics 48 871 1971
27 Schwarcz R L Belizan J M Cifuentes J R Cuadro J C Marques M B & Caldeyro Barcia R Fetal and maternal monitoring in spontaneous labors and in elective inductions Am J Obstet Gynecol 120 356 1974
28 Soule III A B Standley K & Copans S A Clinical uses of the Brazelton Neonatal Scale Pediatrics 54 583 1974
29 Strauss M E Lessen Firestone J K Starr R H & Ostrea E M Behavior of narcotics addicted newborns Child Dev 46 887 1975
30 Tronick E Wise S Als H Adamson L Scanlon J & Brazelton T B Regional obstetric anesthesia and newborn behavior Effect over the first ten days of life Pediatrics 58 94 1976
31 Tylleskär J Finnstrom O Hedenskog S Leijon I & Rydén G Spontaneous labour and elective induction—a prospective randomized study Effects on mother and foetus Acta Obstet Gynecol Scand in press
32 Westin B Gravidogram and foetal growth Comparison with biochemical supervision Acta Obstet Gynecol Scand 56 273 1977

Submitted Nov 16 1978
Accepted Jan 15 1979

(I L) Department of Paediatrics
University Hospital
S 581 85 Linkoping
Sweden

Acta Pædiatr Scand 68 561-566 1979

EFFECT OF INTRAUTERINE NUTRITIONAL DEPRIVATION ON NEUROMOTOR BEHAVIOUR OF THE NEWBORN

V P BHATIA G P KATIYAR and K N AGARWAL

From the Department of Paediatrics Institute of Medical Sciences Banaras Hindu University Varanasi India

ABSTRACT Bhatia V P Katiyar G P and Agarwal K N (Department of Paediatrics Institute of Medical Sciences Varanasi India) Effect of intrauterine nutritional deprivation on neuromotor behaviour of the newborn Acta Paediatr Scand 68 561 1979 —The neurological maturation in 25 newborn babies born to severely undernourished mothers was studied by evaluating muscle tone and excitability status These mothers had weight below the 25th percentile expected for height haemoglobin less than 80 0 g/l and serum albumin less than 25 0 g/l Twenty three babies born to healthy mothers were studied as control The babies of undernourished mothers demonstrated gross intrauterine growth retardation There occurred parallel reduction in placental weight and its protein content The neuromotor behaviour of these newborns showed significant alteration in the performance of most reflexes resembling normal motor behaviour of preterm infants As many as 72% newborns could be classified as hypotonic and 56% hypoexcitable However no newborn demonstrated hypertonia or hyperexcitability The parameters of neuromotor assessment were found to have no correlation with the birth weight in both undernourished as well as control groups These observations suggest that muscle tone and excitability are better indices of maturation of central nervous system than the birth weight It seems that the neurological evaluation becomes unreliable in babies who suffer from intrauterine nutritional deprivation

KEY WORDS Newborn muscle tone muscle excitability maternal undernutrition

Studies on normal preterm and low birth weight newborn babies have led to a general concensus that neurologic maturation proceeds in accordance with the gestational age and is unaffected by premature birth and adverse intrauterine influences (3 10 18 19) This theory has received additional support from studies of electroencephalographic maturation nerve conduction velocity and evoked potentials in the newborn (4 5) However follow up studies of intrauterine growth retarded babies have demonstrated serious motor and intellectual deficiencies suggesting that this concept should be applied cautiously to newborns who have suffered severe intrauterine insult especially nutritional deprivation (2) In the present study muscle tone and excitability status of newborns who suffered from severe intrauterine undernutrition have been assessed by quantitative evaluation of various reflexes

MATERIAL AND METHODS

Subjects

Twenty five newborn babies of severely undernourished mothers who had haemoglobin and serum albumin levels less than 80 0 g/l (mean 50 5 S E ±2 0 g/l) and 25 0 g/l (mean 17 2 S E ±1 0 g/l) respectively and weighed less than the 25th percentile of the expected weight for height (mean 40 8 S E ±0 89 kg) were taken for the study The mean height of these mothers was 149 3±1 06 cm Maternal height and weight were measured 1-2 days after delivery Twenty three newborn babies of healthy mothers having regular antenatal check up haemoglobin more than 100 0 g/l serum albumin more than 35 0 g/l and height and weight above 145 0 cm (155 1±0 97 cm) and 45 0 kg (55 9±1 35 kg) respectively served as controls In both the groups mothers with uncomplicated antenatal and obstetrical history and known date of con

Table 1 *Placental weight protein content and birth weight in control and undernourished groups (mean ± S E)*

n = number of observations *Values in parentheses indicate the range*

Parameters	Control group (*n* = 23)	Under nourished group (*n* = 25)
Placental weight (g)	451±13 0 (397 0–547 0)	299±11 9 • (256 0–400 0)
Placental protein content (mg/g wet tissue)	62 9±1 00 (52 0–68 0)	57 1±1 04 (48 8–68 8)
Birth weight (g)	3 140±71 7 (2 720–3 690)	2 034±50 5 (1 500–2 270)

• $p<0\ 001$

ception only were included Mothers with pathological conditions responsible for blood loss (e g antepartum haemorrhage) proteinuria (e g toxaemia pyelonephritis nephrosis etc) or conditions likely to affect foetal growth e g chronic infections tuberculosis heart disease hypertension and diabetes were excluded from the study All the newborns studied were full term having gestation of 280±2 days with an uneventful delivery and hospital stay Placentae were also studied so as to evaluate the effect of intrauterine undernutrition on the foeto maternal unit

Methodology

A *Neurological assessment* The neurological examination was performed 2–4 days after birth and 1½ to 2½ hours after feeding in a room having a comfortable temperature and humidity None of the neonates had any feeding problems and all were on normal bottle or breast feeding

The method of examination and the scoring was adopted from Prechtl & Beintema (16) Further some complex motor phenomena like spontaneous motor activity of face and general body rooting sucking and cry were also scored on the four point scale as attempted by Schulte (20) All responses were obtained during optimal behaviour state only

Various reflexes and responses in the newborn can be grouped into (*a*) those dependent on phasic neuronal activity and therefore reflecting excitability status of the newborn and (*b*) those dependent on constant neuronal discharge i e related to muscle tone and posture Both excitability and muscle tone status are considered to be important aspects of newborn behaviour indicating maturity of the central nervous system and/or its dysfunction Neurological evaluation was thus conducted from two aspects

(a) *Responses reflecting the muscle tone status of the newborn* These comprised of posture of arms and legs resistance to passive movement of neck trunk jaw arms and legs recoil of forearms traction response posture in prone suspension and supporting reaction

(b) *Reflexes and responses reflecting excitability status of the newborn* These included spontaneous motor activity of general body and face glabellar reflex optical and acoustic blinks biceps triceps knee ankle and jaw jerks palmar and plantar grasps crossed extensor reflex withdrawal rooting sucking Gallant s response Moro s response stepping and cry

The various reflexes and responses were scored as follows

Response	Score
No response	0
Weak response/resistance	1
Good response/resistance	2
Strong/exaggerated response/low threshold/tremulousness	3

An account was also made for asymmetrical responses

The sum of individual scores in each group served as quantitative indices of the excitability and muscle tone status of the newborn Accordingly babies we classified as hypo/hyper-excitable or hypo/hyper tor when their score varied more than ±2 S D from the me excitability and muscle tone scores respectively of t normal newborns

B *Biochemical studies* Maternal blood samples we collected before delivery and serum was separated ir mediately Wet placental weight was recorded aft trimming the membranes to the margin and cutting tl cord at insertion Tissue protein as well as serum album and total proteins were estimated by the method d scribed by Lowry et al (13) Since blood glucose ca cium and magnesium levels affect the neuromotor e citability as well as the EEG pattern these paramete were analysed in the newborns serum immediately aft the recordings were taken The methods adopted f blood sugar (glucose oxidase method) serum magnesiu (Titan yellow method) and serum calcium (Tisdall methc as modified by Clark & Collip) are described by Frank et al (9)

RESULTS

All the newborns studied were apparentl healthy on general and systemic examinatio and none had any congenital anomaly Non suffered from any birth injury or birth anox and their hospital stay (mean 6±3 days) wa uneventful Babies born to undernourishec mothers demonstrated significantly low mean for birth weight placental weight and placen tal protein content as compared to the well nourished group (Table I) The correlation coefficient (*r*) between placental weight and newborn weight were calculated in the con trol and undernourished groups separately

Table 2 *Percentage of babies showing different scores for individual reflexes grouped under Muscle Tone Status*

Values in parentheses indicate the sample size

Reflexes	Control group (23) Score				Undernourished group (25) Score			
	0	1	2	3	0	1	2	3
1 Posture of arms	–	–	100	–	4	36	60	–
Posture of legs	–	–	95.6	4.4	–	36	64	–
Response to passive movement								
3 – Neck	4.4	65.2	30.4	–	20	76	4	–
4 – Trunk	–	30.4	69.6	–	4	84	12	–
5 – Jaw	–	26.1	73.9	–	4	72	24	–
6 – Arms	–	–	65.2	34.8	–	76	24	–
7 – Legs	–	–	34.8	65.2	–	16	84	–
8 Recoil of forearms	–	21.7	60.9	17.4	32	56	12	–
9 Recoil of legs	4.4	–	47.8	47.8	8	0	7	–
10 Traction response	–	17.4	78.3	4.4	40	40	0	–
11 Head control	13.0	6.1	[illegible]	8.7	48	40	12	–
12 Posture in prone suspension	–	13.0	82.6	4.4	20	60	16	4
13 Supporting reaction	–	13.0	56.6	30.4	8	72	12	8

The values were found to be +0.44 ($p<0.01$) and +0.32 ($p<0.01$) respectively. The mean head circumference of the babies belonging to undernourished group was 33.2±0.38 cm which did not differ significantly from control group (34.1±0.27).

Four newborns born to undernourished mothers showed severe intrauterine growth retardation with birth weights of 1500, 1668, 1804 and 1814 g; all were kept in low birth weight nursery for a period of 3 weeks or more. Their stay in nursery was free from any significant illness with satisfactory weight gain.

Table 3 *Percentage of babies showing different scores for individual reflexes grouped under Excitability Status*

Values in parentheses indicate the sample size

Reflexes	Control group (23) Score				Undernourished group (25) Score			
	0	1	2	3	0	1	2	3
1 Spontaneous motor activity (Gen.)	–	4.4	95.7	–	–	32	68	–
2 Spontaneous motor activity (Face)	–	–	100	–	–	4	76	–
3 Glabellar reflex	–	1.7	73.9	4.4	–	56	44	–
4 Optical blink	–	8.7	73.9	17.4	–	60	8	1
5 Acoustic blink	–	13.0	56.5	30.4	–	68	3	–
6 Biceps jerk	–	30.4	69.6	–	–	68	3	–
7 Triceps jerk	4.4	60.9	34.8	–	16	84	–	–
8 Knee jerk	–	4.4	95.7	–	–	24	72	4
9 Palmar grasp	–	8.7	73.9	17.4	–	24	64	12
10 Plantar grasp	4.4	4.4	65	26.1	–	44	44	1
11 Crossed extensor reflex	–	43.5	47.8	8.7	–	72	0	8
12 Withdrawal reflex	–	4.4	95.7	–	–	24	68	8
13 Rooting response	–	13.0	78.3	8.7	–	5	44	4
14 Sucking	–	4.4	86.9	8.7	–	24	7	4
15 Jaw jerk	–	47.8	52.2	–	–	64	36	–
16 Moro response	–	–	78.3	21.7	–	28	64	8
17 Ankle jerk	–	56.5	43.5	–	–	60	40	–
18 Stepping	–	34.8	56.5	8.7	8	64	24	4
19 Gallant's reflex	4.4	39.1	56.5	–	16	48	36	–
20 Cry	–	–	17	78.3	–	16	7	12

Table 4 *Muscle tone and excitability scores in babies born to control and undernourished mothers (mean±S E)*

Values in parentheses indicate the sample size

Group	Control (23)	Under nourished (25)
Muscle tone score	25 74±0 74	16 24±0 62 **
Excitability score	37 87±0 81	30 68±0 86
Modal muscle tone score	27	15
Modal excitability score	39	30

** $p<0\ 001$

Neurological behaviour

(i) *Muscle tone status* The newborns of the undernourished group demonstrated poor response to most of the reflexes which reflect Muscle tone status and 40% showed partial to complete limp posture as compared to the control newborns The lower limbs were observed to be less affected in terms of passive resistance and recoil The reduced tone of neck trunk arms and jaw resulted in an absence of head control in 48% and limp head was found during prone suspension in 80% as compared to corresponding figures of 13% for both these reflexes in the control group The response to supporting reaction revealed that axial muscle tone was reduced in the undernourished group (Table 2)

The muscle tone score for the newborns of the undernourished group was significantly lower than the control group ($p<0\ 001$ Table 4) The modal muscle tone scores for the control and undernourished groups were 27 and 15 respectively On the basis of these scores 18 (72%) newborns belonging to the undernourished group could be classified as hypotonic (score less than 18)

(ii) *Excitability status* One third to one fourth of the newborns in the undernourished group showed poor spontaneous motor activity in general and facial activity as compared to that observed in the control group The babies born to undernourished mothers had poor performance for almost all the reflexes grouped for evaluating excitability However various tendon reflexes (biceps triceps knee and jaw jerks) Gallant s response and cry were less affected In 28% of the newborns belonging to the undernourished group Moro s response was incomplete with an absent or weak adductor phase Except for knee jerk other tendon jerks were difficult to elicit in a fairly large number of control newborns as well (Table 3)

The calculated excitability scores were 37 9±0 81 and 30 7±0 86 respectively for newborns of the control and undernourished groups the difference being significant ($p<0\ 001$) The modal scores were found to be 39 and 30 for these groups respectively On this basis 14 (56%) newborns of undernourished mothers had excitability score less than 2 S D of the mean score of the control group (score 30) and therefore were considered hypoexcitable or apathetic No newborn was found to be hyperexcitable (Table 4)

The muscle tone and excitability scores in different birth weight groups demonstrated identical means for muscle tone and excitability scores for the newborns weighing between 2 000–2 500 g and those below 2 000 g (Table 5) However the means in both groups were lower than the means observed in newborns above 2 500 g birth weight The levels of blood sugar serum calcium and magnesium were found to be identical in newborns of both groups

DISCUSSION

In this study babies of severely undernourished mothers showed intrauterine growth

Table 5 *Distribution of muscle tone and excitability scores according to birth weight (mean ±S E)*

Values in parentheses indicate mean weights

Birth weight (g)	n	Muscle tone score	Excitability score
>2 500 (3 140)	23	25 74±0 74	37 87±0 81
2 000–2 500 (2 146)	13	16 69±0 92	31 42±1 47
<2 000 (1 870)	12	15 75±0 88*	30 0±1 04

*** $p<0\ 001$

retardation as evident by the findings of low birth weight and reduced placental size This has been observed by other workers and ascribed to reduced protein biosynthesis (12 21 22)

Neurological status

Available studies have demonstrated that the neuromotor development of the newborn proceeds in accordance with the conceptional age and remains unaffected in preterm or small for dates babies Graziani et al (11) selected a group of reflexes which mature during early organogenesis and are not likely to be affected by nutritional deprivation or factors operative in the later part of pregnancy It is also likely that simple studies of reflex patterns eliciting their presence may not indicate the maturity status since responses may be qualitatively modified Further studies have not classified their group of low birth weight infants and therefore not only the cause–effect relationship is lost but some otherwise normal low birth weight infants get included along with pathologically growth retarded neonates (6 18 19)

Prechtl (17) correlated the obstetrical risk factors with the neuromotor development and concluded that the mean neurological score decreased as the number of obstetrical complications increased Farr & Mitchell (7) and Michaelis et al (14) also demonstrated altered neuromotor maturation in low birth weight babies The studies in babies of toxaemic mothers known to suffer pathological intrauterine growth retardation demonstrated significant hypotonia in these newborns (20) These workers were of the opinion that neuromotor maturation is likely to be affected if the materno-foetal supply line is jeopardized in any pathological condition

In the present study babies of severely undernourished mothers on assessment for quality of the responses and the cumulative scores for various grouped reflexes demonstrated (i) significant hypotonia (72%) (ii) marked hypoexcitability (56%) and (iii) modification of responses in several reflexes viz limp posture poor recoil of limbs incomplete Moro s and crossed extensor responses etc as compared to the babies born to well nourished mothers These features observed in undernourished newborns were close to the observations made by earlier workers in preterm babies (1 6 19) However it is difficult to say whether associated anaemia along with generalised protein-energy deprivation also affects neuromotor maturation by chronic intrauterine hypoxia

The muscle tone and excitability scores were similar amongst the babies weighing less than 2 000 g and those weighing between 2 000–2 500 g (Table 5) When these scores were analysed in relation to the birth weight gestation being full term in all the newborns studied no relation could be demonstrated between the two though both neuromotor development and birth weights were retarded This suggests that evaluation of muscle tone and excitability are better indices when assessing the maturity of the central nervous system

The critical analysis of neuromotor behaviour of the newborns who suffered from intrauterine undernutrition revealed that the reflexes mediated by polysynaptic pathways viz optical and acoustic blinks crossed extensor reflex rooting sucking Moro s and stepping responses were affected much more than those reflexes mediated through monosynaptic pathways e g various tendon reflexes Muscle tone also is dependent on a complex polysynaptic neural system involving cerebellum reticular formation and various related nuclei Animal studies have demostrated defective cerebellar growth deficient dendritic arborization and alterations in reticular formation (8 15) These observations offer a possible explanation for the above results

The present study concludes that intrauterine undernutrition results in altered activity of the brain as evident by muscle tone and excitability status So far no study is available on severe maternal undernutrition and other authors have taken small for-dates babies

from pregnancy disorders like toxaemia anaemia due to antepartum haemorrhage smoking twinning etc (2 7 14 17 20) Still the concensus emerges that retardation of neuromotor maturation occurs during altered pregnancy states and maternal undernutrition Therefore any assessment of neurological status to evaluate the gestational age in such babies is likely to be fallacious

REFERENCES

1 André Thomas Chesni Y & St Anne Dargassies S The neurological examination of the infant *Clinics in Developmental Medicine No 1* Spastics Society Heinemann London 1960

2 Bhakoo O N Kaur S Narang A Verma S K Chopra I S & Gupta A N Neonatal neurological examination perinatal factors subsequent development and evaluation of a prognostic risk score *Indian J Pediatr 14* 263 1977

3 Dotson E & Desmond M M The evaluation of muscle tonus in the newborn *Neurology (Minneap) 14* 464 1964

4 Dreyfus Brisac C & Minkowski A Electroencephalographic maturation and low birth weight *In* P Kellaway and I Peterson (eds) *Neurological and EEG correlative studies in infancy* Grune and Stratton New York 1964 p 49

5 Ellingson R J & Lindsley D B Brunwaves and cortical development in newborns and young infants *Am Psychol 4* 248 1964

6 Farr V Estimation of gestational age by neurological assessment in first week of life *Arch Dis Child 43* 353 1968

7 Farr V & Mitchell R G Effect of birth weight on maturity scoring *Develop Med Child Neurol 9* 745 1967

8 Fish I & Winick M The effect of malnutrition on the regional growth of developing rat brain *Exper Neurol 25* 534 1969

9 Frankel S Reitman S & Sennenwirth A C *Gradwhol s Clinical laboratory methods and diagnosis* C V Mosby Company St Louis 1970 7th edition

10 Gesell A A & Amatruda C S *In* H Knobloch and B Pasamanick (eds) *Developmental Diagnosis* Harper and Row Publishers New York 1974 3rd edition

11 Graziani L J Weitzman E D & Velasco M S Neurologic maturation and auditory evoked responses in low birth weight infants *Pediatrics 41* 483 1968

12 Laga E M Driscoll S G & Munro H N Comparison of placentas from two socioeconomic groups I Morphometry *Pediatrics 50* 24 1972

13 Lowry O H Rosenbrough N J Farr A L & Randall R J Protein measurement with Folin phenol reagent *J Biol Chem 193* 265 1951

14 Michaelis R Schulte F J & Nolte R Motor behaviour of small for gestational age newborn infants *J Pediatr 76* 208 1970

15 Platt B S Heard C R C & Steward R J C Experimental protein calorie deficiency *In* H R Munro and J B Allison (eds) *Mammalian protein metabolism* Academic Press New York 1964 vol p 446

16 Prechtl H F R & Beintema D Neurological examination of full term newborn infant *Clinics in Developmental Medicine* No 12 Spastics Society Heinemann London 1964

17 Prechtl H F R Neurological sequelae of prenatal and perinatal complications *Br Med J 11* 763 1967

18 Robinson R J Assessment of gestational age by neurological examination *Arch Dis Child 41* 437 1966

19 Saint Anne Dargassies S La maturation neurologique du prématuré *Etud Neonatal 4* 71 1955

20 Schulte F J Maternal toxaemia foetal malnutrition and motor behaviour of the newborn *Pediatrics 48* 871 1971

21 Sen S & Agarwal K N Placental alterations in relation to maternal socio-economic status *Indian Pediatr 12* 865 1975

22 Winick M Cellular growth of human placenta III Intrauterine growth failure *J Pediatr 71* 390 1967

Submitted Oct 4 1978
Accepted Dec 13 1978

(G P K) Department of Paediatrics
Institute of Medical Sciences
Banaras Hindu University
Varanasi
India

EVIDENCE OF RIBOFLAVIN DEPLETION IN BREAST FED NEWBORNS AND ITS FURTHER ACCELERATION DURING TREATMENT OF HYPERBILIRUBINEMIA BY PHOTOTHERAPY

L HOVI R HEKALI and M A SIIMES

From the Children s Hospital University of Helsinki and Finnish Blood Transfusion Service Helsinki Finland

ABSTRACT Hovi L Hekali R and Siimes M A (Children s Hospital University of Helsinki and Finnish Blood Transfusion Service Helsinki Finland) Evidence of riboflavin depletion in breast fed newborns and its further acceleration during treatment of hyper bilirubinemia by phototherapy Acta Paediatr Scand 68 567 1979 —Phototherapy in the treatment of newborns with hyperbilirubinemia resulting in degradation of bilirubin also appears to have other photodynamic effects on metabolism We studied flavin adenine dinucleotide (FAD) saturation of erythrocyte glutathione reductase which should reflect riboflavin nutritional status in 28 healthy newborns and followed 37 newborns with hyper bilirubinemia prior to the start of and during phototherapy The results indicate that healthy newborns on human milk feeding relatively poor in riboflavin have evidence of a transient riboflavin depletion soon after birth This effect is made more pronounced by photo therapy and partially prevented by parenteral or oral administration of moderate amounts of riboflavin

KEY WORDS Breast feeding hyperbilirubinemia newborns phototherapy red blood cell enzymes riboflavin depletion

Phototherapy has an established role in the treatment of newborns with hyperbilirubinemia (21) This kind of exposure to high intensity light resulting in degradation of bilirubin also appears to have other less beneficial photodynamic effects on metabolism Mild symptoms and signs are reported to be surprisingly common in jaundiced newborns receiving phototherapy including fever diarrhea irritability and skin rash (8) Fever and exposure of skin have been related to potential dehydration (16 22) acid base balance disturbances (15) and subsequent circulatory responses (17) Diarrhea has been associated with deficiencies of intestinal enzymes such as disaccharidase (6) and lactase (1) Effects have also been reported on metabolism of nuclear DNA (20) and red blood cells (3) and even on the blood concentrations of various constituents including platelets (14) lipids (11) and several hepatic enzymes (5 18) However some of the above mentioned findings have only been observed in low birth weight infants or in animal studies Some long term sequelae related to growth and development have been suggested although these findings have been controversial (8) However a bronze baby syndrome (12) and visual complications (7) are well documented although the latter are preventable by simple means Recent data also indicate that infants with hemolytic disease such as glucose 6 phosphate dehydrogenase deficiency may not respond to phototherapy and increased hemolysis may result from the treatment (13)

Potentially correctable side effects of phototherapy are for example those which result in deficiencies of light sensitive vitamins such as riboflavin (10 24) In this study we found some evidence of transient riboflavin depletion both in healthy and jaundiced newborns fed with breast milk during the first weeks of life and explored further the evidence of riboflavin depletion in jaundiced newborns during phototherapy and its prevention by administration of parenteral or oral riboflavin

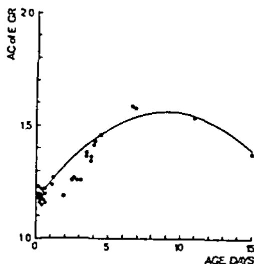


Fig 1 Evidence of transient riboflavin depletion in healthy newborns and in newborns with hyperbilirubinemia prior to the start of phototherapy Significance of the correlation between activity of erythrocyte glutathione reductase (AC of E GR) and age was high ($p<0.001$)

SUBJECTS AND METHODS

The subjects were 37 consecutive full term newborns who were referred to the Helsinki Children s Hospital with hyperbilirubinemia (over 170 µmol/l (10 mg/100 ml)) but without evidence of a hemolytic disease or other major medical problem In addition 28 healthy newborns were studied from 0 to 14 days as controls

The newborns with hyperbilirubinemia were treated by phototherapy for periods from 1 to 7 days The therapy was started at an average age of 3 5 days with a total range from 1 to 8 days Two newborns were subsequently exchange transfused after which they were excluded from these data

During phototherapy the newborns were undressed and only their eyes were shielded There was a continuous exposure to light except during nursing A bank of six natural white bulbs (Airam IXC) each of 20 W were kept at a distance of 35 cm above the baby Each bulb was tested for its intensity at the intervals of three months and replaced if the intensity of visible light was less than 4 000 lux No additional shields were used since the lamps yielded only a trace amount of ultraviolet irradiation

All subjects were fed with banked or fresh breast milk from birth through the period of the study

The first 10 of the 37 newborns with hyperbilirubinemia received no riboflavin supplementation during phototherapy Subsequently they randomly received riboflavin either intramuscularly ($n=8$) or orally ($n=10$) or no supplementation ($n=9$) Thus a total group of 19 newborns was not treated with riboflavin The vitamin (riboflavin Na phosphate) was administered twice a day at a dose of 0 5 mg/kg/day The administration was started immediately or within 8 hours after the initiation of phototherapy and continued through its duration

Flavin adenine dinucleotide (FAD) saturation of erythrocyte glutathione reductase (E GR) is considered a specific indicator of riboflavin nutritional status (4) A total of 54 blood samples was obtained from 37 newborns with *hyperbilirubinemia prior to the start of phototherapy* In addition 94 samples were drawn at about 4 hour intervals during phototherapy to check the E-GR About half of the latter ($n=49$) were from newborns receiving riboflavin treatment The group of healthy newborns was sampled only once The blood was drawn in ACD solution kept at 4 C until centrifucation and washed twice with isotonic saline solution The hemolysate was kept at −20°C until analyzed (?) The tests with and without FAD were performed concurrently The results were expressed as activity coefficients (AC) where values above 1 0 indicate riboflavin deficiency (4)

The correlations were calculated by parabolic regressions Statistical significances were also determined by Student s *t* test

RESULTS

The AC values of E GR of the healthy newborns and of the newborns with hyperbilirubinemia prior to the start of phototherapy did *not differ from one another and were thus used as control values* $n=82$ *(Fig 1)* Accordingly hyperbilirubinemia per se had no influence on the activity of the enzyme However the values were age dependent and increased during the first week of life This rise reached statistical significance ($p<0.001$) The mean values were slightly lower again during the second week of life as shown by *the regression line in Fig 1*

Phototherapy for hyperbilirubinemia further accentuated the increase in the AC values

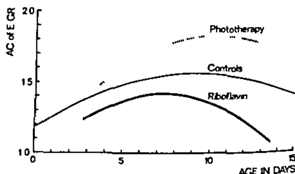


Fig 2 Further acceleration of riboflavin depletion after initiation of phototherapy in newborns with hyperbilirubinemia and its prevention by riboflavin treatment Significances of the differencies are indicated in the text

(Fig 2) This difference reached statistical significance over the control values after 5 days of age ($p<0.0025$) The effect was dependent on the duration of phototherapy ($p<0.0025$) although it became pronounced within 24 hours The mean values increased further (by the nonlinear regression line) from 1 52 at 24 hours to a maximum of 1 74 at 4 days of phototherapy respectively

The treatment with riboflavin resulted in a marked decrease in the AC values ($p<0.001$) to mean levels which were below the control values at any age (Fig 2) This effect was similar in the newborns who were treated either intramuscularly or orally although the mean values of the former group were slightly (about 0 2 AC units) but insignificantly lower after 6 days of age For these reasons the values of both treatment groups were pooled in Fig 2

DISCUSSION

The results show that the AC values of E GR increased in any group of newborns during the first week of life and that the newborns with hyperbilirubinemia receiving phototherapy showed a further increase This effect was partially prevented by additional treatment with moderate amounts of riboflavin administered either parenterally or orally Only these AC values reached a mean of 1 0 by two weeks of age a value which should represent a normal mean These observations suggest that phototherapy results in evidence of riboflavin depletion and confirm and further extend those reported by Gromisch and his associates (10)

In addition we found that even in healthy newborns there was some evidence of a transient depletion soon after birth It is of interest that this finding might be explained by the exclusive feeding of human milk in our subjects since the concentration of riboflavin is relatively low in human milk about 0 36 mg/l (20) In the two previous studies the authors did not report this phenomenon (10 24) However their subjects were fed with infant milk formulas which contain about three times more riboflavin than human milk These considerations indicate that newborns on human milk feeding might be more sensitive to the phototherapy induced riboflavin depletion Further the level of riboflavin in human milk may be marginal for the needs of every healthy newborn soon after birth

Babies under phototherapy had no distinct symptoms or signs which could have been correlated with the degree of enzyme activity However flavin nucleotides serve as coenzymes in several major metabolic pathways Thus it remains possible that phototherapy might cause harmful metabolic changes In accord with this possibility there is evidence for altered tryptophan metabolism during phototherapy probably through riboflavin deficiency (19) It is not known however whether other metabolic pathways requiring FAD are affected for instance the catabolism of purine nucleotides

Further information is also needed to determine whether riboflavin supplementation might be beneficial in healthy human milk fed newborns and in those receiving phototherapy A potential disadvantage of riboflavin treatment is suggested by *in vitro* studies which indicate that light exposed riboflavin may also have potential side effects (23)

In many hospitals phototherapy is liberally administered and only a few newborns with some hyperbilirubinemia may avoid it However the numerous reports including our findings tend to indicate various potential side effects It remains to be determined whether the risks outweigh the benefits in most of the infants subjected to treatment

REFERENCES

1 Bakken A F Intestinal lactase deficiency as a factor in the diarrhea of light treated jaundiced infants *N Engl J Med* 294 615 1976
2 Beutler E Blume K G Kaplan J C Lohr G M Ramot B & Valentine W N International Committee for Standardization in Haematology Re

commended methods for red cell enzyme analysis *Br J Haematol 35* 331 1977
3 Blackburn M G Orzalesi M M & Pigram P Effect of light on fetal red blood cells *in vivo J Pediatr 80* 640 1972
4 Cooperman J M Cole H S Gordon M & Lopez R Erythrocyte glutathione reductase as a measure of riboflavin nutritional status of pregnant women and newborns *Proc Soc Exp Biol Med 143* 326 1975
5 Dacou Voutetakis C & Anagnostakis D Phototherapy and serum LH levels in newborns *Pediatr Res 10* 876 1976
6 Dinari G Daum F Cohen M I & McNamara H Effect of phototherapy on intestinal disaccharidase activity in the rat *Pediatr Res 11* 443 1977
7 Dobson V Cowett R M & Riggs L A Long term effect of phototherapy on visual function *J Pediatr 86* 555 1975
6 Drew J H Marriage K J Bayle V V Bajraszewski E & McNamara J M Phototherapy Short and long term complications *Arch Dis Child 51* 454 1976
9 Fomon S J & Filer L J Milk and formulas *In* S J Fomon (ed) *Infant nutrition* W B Saunders New York 1974 2nd ed p 359
10 Gromisch D S Lopez R Cole H S & Cooperman J M Light (phototherapy)-induced riboflavin deficiency in the neonate *J Pediatr 90* 118 1977
11 Hadjigeorgiou E Trihouri D Trichopoulou A Kaskarellis D & Nicolopoulos D Influence of phototherapy on the serum lipids of jaundiced new born infants *Pediatr Res 12* 690 1978
12 Kopelman A E Brown R S & Odell G B The bronze baby syndrome A complication of phototherapy *J Pediatr 81* 466 1972
13 Kopelman A E Ey J L & Lee H Phototherapy in newborn infants with glucose 6-phosphate dehydrogenase deficiency *J Pediatr 93* 497 1978
14 Mauer H M Fratlin M McWilliams N B Kirkpatrick B Draper D Haggins J C & Hunter C R Effect of phototherapy on platelet counts in low birth weight infants and on platelet production and life span in rabbits *Pediatrics 57* 506 1976
15 Mueller D G Kirkpatrick B V Stoerner J W Hunter C R & Mauer H M Effect of phototherapy on acid base balance in low birth weight infants *Pediatr Res 10* 478 1976
16 Oh W & Karecki H Phototherapy and insensible water loss in the newborn infant *Am J Dis Child 124* 230 1972
17 Oh W Yao A C Hanson J S & Lind J Peripheral circulator response to phototherapy in newborn infants *Acta Paediatr Scand 62* 49 1973
18 Orzalesi M Natoli G Panero A & Ciocca M Plasma hepatic enzymes in jaundiced newborn infants treated with phototherapy *In* D Bergsma & S H Blondheim (eds) *Bilirubin Metabolism in the Newborn* II Birth Defects Original Article Series vol 12 no 2 Williams & Wilkins Baltimore 1976 p 93
19 Rubaltelli F F Allegri G Costa C & De Antoni A Urinary excretion of tryptophan metabolites during phototherapy *J Pediatr 85* 865 1974
20 Santella R M Rosenkranz H S & Speck W T Intracellular deoxyribonucleic acid modifying activity of intermittent phototherapy *J Pediatr 93* 106 1978
21 Seligman J W Recent and changing concepts of hyperbilirubinemia and its management in the newborn *Pediatr Clin North Am 24* 509 1977
22 Smales O R C Effect of phototherapy on thermal environment of the newborn *Arch Dis Child 53* 172 1978
23 Speck W T Chen C C & Rosenkranz H S *In vitro* studies of effects of light and riboflavin on DNA and HeLa cells *Pediatr Res 9* 150 1975
24 Tan K L Chow M T & Karim S M M Effect of phototherapy on neonatal riboflavin status *J Pediatr 93* 494 1978

Submitted Oct 9 1978
Accepted Dec 18 1978

(M A S) Children s Hospital
University of Helsinki
Stenbackinkatu 11
SF 00290 Helsinki 29
Finland

Acta Pædiatr Scand 68 571–573 1979

A LONGITUDINAL STUDY OF MANGANESE IN HUMAN MILK

E VUORI

From the Department of Public Health Science
University of Helsinki Helsinki Finland

ABSTRACT Vuori E (Department of Public Health Science University of Helsinki Helsinki Finland) A longitudinal study of manganese in human milk Acta Paediatr Scand 68 571 1979 —Flameless atomic absorption spectrophotometry has been applied to the analysis of manganese in human milk samples Twenty seven breast feeding mothers donated 2 9 individual milk samples between the 2nd week and the 9th month of lactation The milk samples were representative of every feed in a period of 24 hours foremilk and hind milk in equal proportions The median concentration of manganese declined from the initial value of 5 9 μg/l to about 4 μg/l before the 2nd month of lactation remained at this level up to the 5th–6th month of lactation and showed a tendency to rise thereafter The values presented here are noticeably lower than most of those reported earlier It remains an open question whether the manganese concentration is exceptionally low in Finnish human milk or whether the great difference from most earlier studies may reflect problems of contamination or technical difficulties when less sensitive analytical methods have been used

Key words milk human manganese

Compared with other trace elements such as iron copper and zinc the number of studies concerning the manganese concentration of human milk is small The mean manganese concentration has ranged from 7 to 120 μg/l (2 5–7) Any variation to this extent in the average value of an essential trace element is not likely to be a physiological phenomenon So far no data is available to illustrate how the manganese concentration of human milk varies with the stage of lactation The discrepant values on the manganese levels in human milk are also reflected in the recommendation for manganese in different milk formulas in 1971 The Codex committee recommended the minimum manganese concentration in complete infant formula to be 200 μg/100 kcal but in 1976 this figure was changed to 5 μg/100 kcal (1 2) More detailed data are obviously needed on the manganese concentration of human milk in order to achieve unanimity in this question

SUBJECTS AND METHODS

Twenty seven breast feeding mothers supplied ?9 milk samples between the nd week and the 9th month of lactation At first the mothers delivered samples at ? week intervals and after the ?nd month at intervals of 3–4 weeks Each individual milk sample was representative of every feed during a period of 4 hours and consisted of equal volumes of foremilk and hindmilk (10) The present method of collecting milk samples allowed the mother to continue breast feeding without interruption and minimized the potential diurnal variation and any variation in the composition of the milk within a single feed The number of breast feeding mothers declined during the follow up being 23 13 and ? in the 3rd 6th and 9th month of lactation respectively The group of mothers and the method of collecting milk samples have been discussed earlier in detail (10)

After dry ashing at 450 C (10) the manganese concentration was determined from ash solutions with flameless atomic absorption spectrophotometry (Perkin Elmer model 300 with HGA 100) using deuterium background correction Since it has been shown that calcium and magnesium can interfere with manganese determinations when the flameless technique is used (9) the influence of the major cations in concentrations present in mature human milk (4) on manganese standards was tested but no effect was found The accuracy of the method was studied by analysing Bovine Liver Standard Reference material No 1577 National Bureau of Standards (NBS) Washington D C Our mean result from four determinations was 11 0 μg/g dry weight with a range of 10 8–11 ? μg/g The certified value for manganese is 10 3±1 0 μg/g as given by NBS The precision of the method was established by repeated analysis of a pooled human milk sample The mean results of 11 determinations was 6 4 μg/l S D 0 5 between series

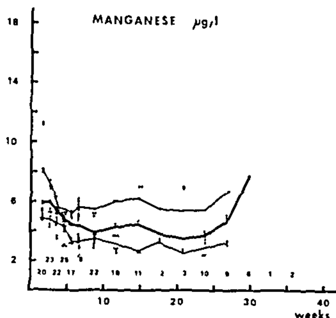


Fig 1 The average manganese concentration of human milk during lactation showing the total range median values and the 25th and the 75th percentiles after the 2nd week at weekly intervals and after the 7th week thrice weekly Total number of samples is 229 Numerals indicate the number of samples at each interval of time

RESULTS AND DISCUSSION

The average values are expressed here by medians and the variation by giving the total range the 25th and 75th percentiles since mean values and standard deviations over estimate the average concentration and variation due to the skewed distribution of values (Fig 1) The median manganese concentration was 5 9 µg/l in the 2nd week of lactation and 50% of the values ranged between 4 9 and 7 0 µg/l The median value declined to about 4 µg/l before the 2nd month of lactation and remained at that level up to the 5th–6th month of lactation where after it showed a tendency to increase (Fig 1) The lowest and highest individual values for manganese in human milk were 1 8 and 17 8 µg/l respectively

Fomon s textbook on pediatric nutrition gives values of 7–15 µg/l as the normal level of manganese in mature human milk (4) The figures given are based on studies where only a few milk samples had been studied and the samples represent only the early stages of lactation (Table 1) The present results are noticeably lower than most of those reported earlier but are comparable with the results of the oldest study presented in Table 1

When the effect of the stage of lactation on the manganese concentration of breast milk was studied it was found that manganese initially showed high values then decreased to a minimum level and finally showed a rising tendency during the last months of the study This pattern differs from that found in the case of zinc where decreasing values were noted throughout the course of lactation (10) and in the cases of copper and iron where a minimum level was found after the 4th–5th month of lactation (8 10)

It remains an open question whether the manganese concentration is exceptionally low in Finnish human milk or whether the great difference from most earlier studies may reflect contamination problems or technical difficulties when less sensitive analytical methods have been used

Table 1 *The average manganese concentration of human milk according to different autho*

Mn (µg/l)	No of samples	Stage of lactation	Reference
7	Several	?	Broek & Wolff 1935 (3)
19	30	6th–7th day	Grebennikov et al 1961 (5)
120	22	?	Murthy & Rhea 1971 (7)
15	10	after 1st week	McLeod & Robinson 1972 (6)
5 9[b]		2nd week	Present study
7 8[b]	229	31st week	

The authors give ppm in ash
[b] Median value

ACKNOWLEDGEMENT

This work was supported by a grant from the Yrjo Jahnsson Foundation Helsinki Finland

REFERENCES

1 ALINORM 71/ 6 Appendix IV 1971
ALINORM 76/ 6 Appendix III 1976

3 Broek A & Wolff L K The quantity of Cu Zn and Mn in cow s milk *Acta Brevia Neer Physi l Pha macol Microbiol* 5 80 1935

4 Fomon S J *Infant Nutrition* W B Saunders Company Philadelphia 1974 nd edn

5 Grebennikov E P Soroka V R & Sabadash E V Trace element in human and animal milk *Fed Pro* 23 461 1964

6 M l eod B E & Robinson M F Dietary intake of manganese by New Zealand infants during the first six months of life *Br J Nutr* 27 229 1972

7 Murthy G K & Rhea U S Cadmium copper iron lead manganese and zinc in evaporated milk infant products and human milk *J Dairy Sci* 54 1001 1971

8 Siimes M A Vuori E & Kuitunen P Breast milk iron – a declining concentration during the ourse of lactation *Acta Paedi itr Scand* 68 29 1979

9 Smeyers Verbeke J Michotte Y Van den Winkel P & Massart D Matrix effects in the determination of copper and manganese in biological materials using carbon furnace atomic absorption spectrometry *Anal Chem* 48 125 1976

10 Vuori E & Kuitunen P The concentrations of copper and zinc in human milk *Acta Paediatr Scand* 68 33 1979

Submitted Nov 2 1978
Accepted Jan 17 1979

Department of Public Health Science
University of Helsinki
Haartmaninkatu 3
SF-00 90 Helsinki 9
Finland

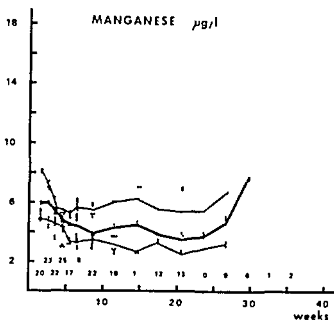


Fig. 1. The average manganese concentration of human milk during lactation, showing the total range, median values and the 25th and the 75th percentiles after the 2nd week at weekly intervals and after the 7th week thrice weekly. Total number of samples is 229. Numerals indicate the number of samples at each interval of time.

RESULTS AND DISCUSSION

The average values are expressed here by medians and the variation by giving the total range, the 25th and 75th percentiles, since mean values and standard deviations overestimate the average concentration and variation due to the skewed distribution of values (Fig. 1). The median manganese concentration was 5.9 µg/l in the 2nd week of lactation and 50% of the values ranged between 4.9 and 7.0 µg/l. The median value declined to about 4 µg/l before the 2nd month of lactation and remained at that level up to the 5th–6th month of lactation, whereafter it showed a tendency to increase (Fig. 1). The lowest and highest individual values for manganese in human milk were 1.8 and 17.8 µg/l, respectively.

Fomon's textbook on pediatric nutrition gives values of 7–15 µg/l as the normal level of manganese in mature human milk (4). The figures given are based on studies where only a few milk samples had been studied and the samples represent only the early stages of lactation (Table 1). The present results are noticeably lower than most of those reported earlier, but are comparable with the results of the oldest study presented in Table 1.

When the effect of the stage of lactation on the manganese concentration of breast milk was studied, it was found that manganese initially showed high values, then decreased to a minimum level and finally showed a rising tendency during the last months of the study. This pattern differs from that found in the case of zinc, where decreasing values were noted throughout the course of lactation (10), and in the cases of copper and iron, where a minimum level was found after the 4th–5th month of lactation (8, 10).

It remains an open question whether the manganese concentration is exceptionally low in Finnish human milk or whether the great difference from most earlier studies may reflect contamination problems or technical difficulties when less sensitive analytical methods have been used.

Table 1. *The average manganese concentration of human milk according to different authors*

Mn (µg/l)	No. of samples	Stage of lactation	Reference
7	Several	?	Broek & Wolff 1935 (3)
19	30	6th–7th day	Grebennikov et al. 1961 (5)
120	22	?	Murthy & Rhea 1971 (7)
15	10	after 1st week	McLeod & Robinson 1972 (6)
5.9[b]		2nd week	Present study
7.8[b]	229	31st week	

[a] The authors give ppm in ash.
[b] Median value.

Acta Pædiatr Scand 68 575–581 1979

UMBILICAL ARTERY CATHETERIZATION IN NEWBORNS

I Thrombosis in Relation to Catheter Type and Position

GÖRAN WESSTRÖM ORVAR FINNSTRÖM and GÖRAN STENPORT

From the Departments of Paediatrics and Diagnostic Radiology University Hospital Linköping Sweden

ABSTRACT Wesstrom G Finnstrom O and Stenport G (Departments of Paediatrics and Diagnostic Radiology University Hospital Linkoping Sweden) Umbilical artery catheterization in newborns I Thrombosis in relation to catheter type and position Acta Paediatr Scand 68 575 1979 Seventy-one sick newborn infants who had an umbilical artery catheterized were randomized in one of four catheter groups long end hole- short end hole- long side-hole or short side hole catheter A long catheter means a high position of the catheter tip (Th) and a short catheter a low position of the tip (L_3) An angiography through the indwelling catheter in order to diagnose thrombosis was performed before the catheter was withdrawn Dissection of the aorta and its branches was performed on infants who died The total frequency of thromboses was 26% There were no thromboses among infants with long end hole catheters while infants with short end hole catheters had thrombosis in 26% long side-hole catheters in 33% and short side-hole catheters in 64% Long end hole catheters functioned better than the others Only 6 of 16 infants with thrombosis had physical signs from the legs while 12 infants without thrombosis had similar signs

KEY WORDS Newborn infant umbilical artery catheterization thrombosis aortography

The use of prolonged umbilical vessel catheterization for the diagnosis and treatment of sick newborns is routine in most paediatric departments In Sweden today roughly 1 6% of all newborn infants have an umbilical artery catheter introduced The figure is based on a nationwide survey (7) Critically ill infants who are receiving oxygen therapy need careful monitoring of arterial oxygen tension (P_O) to allow adequate oxygenation and to reduce the threat of retrolental fibroplasia Arterialized capillary blood is not suitable (14) Other procedures to obtain arterial blood than from the umbilical artery have been recommended but are less convenient Therefore at present catheterization of the umbilical artery remains the standard method for obtaining arterial blood However a variety of complications arising from this procedure has been described Complications previously reported have included embolism thrombosis vascular perforation ischemic necrosis of abdominal viscera infection hemorrhage and blanching of the legs (2 5 9 15 17 23)

The most common serious complication has been thrombosis The rate varies widely in previously reported retrospective (5 9 16 33) and prospective investigations (Table 1) The varying results have led to different recommendations to decrease the risks with regard to the type of catheter the position of the catheter tip as well as the technique for catheterization (13 19 20)

The present prospective study was aimed at elucidating the following questions

– the frequency of thrombosis associated with arterial umbilical catheterization

– the frequency of thrombosis with two different types of catheters and two different positions of the catheter tip

– the most suitable type and position of the catheter for good function

– the correlation between physical signs and thrombosis

Table 3 *The occurrence of thrombosis in relation to type and position of the umbilical artery catheter*

In ? infants both angiography and autopsy were performed

Type and position of catheter	Infants (*n*)	Infants with angiography (*n*)	Thrombosis at angiography (*n*)	Infants with autopsy (*n*)	Thrombosis at autopsy (*n*)	Total number of thrombosis *n*	Total number of thrombosis %	Drop outs (*n*)
Long end hole	0	14	0	6	0	0	0	2
Short end hole	19	15	3	5	?	5	26 3	4
Long side hole	1?	11	4	1	0	4	33 3	1
Short side hole	11	11	7	1	0	7	63 6	-
Total	6	51	14	13	2	16	25 8	9

was registered as well as clinical signs that could be attributed to the catheterization

The investigation was approved by the ethical committee of the medical faculty of the University of Linkoping

RESULTS

The distribution of the 62 infants as well as the number of thromboses within the four groups is shown in Table 3 Sixteen infants (26%) had thrombosis or embolus Fourteen were diagnosed at angiography and 2 at autopsy Thrombosis was present in all sub groups except LE catheter The difference in number of thromboses between long and short catheters (4 and 12) was significant ($p<0\ 05$) as well as the difference between end and side hole catheters (5 and 11) ($p<0\ 01$) There was also a significant difference between LE and LS catheters ($p<0\ 05$) and between LE and SE catheters ($p<0\ 05$) The most pronounced difference ($p<0\ 001$) was found between LE and SS catheters There was some difference though not significant between these four groups in the duration of catheterization as calculated from the introduction of the catheter until angiography (Table 4) Including the infants who died in whom the duration of catheterization was calculated until the moment the infant died there was significantly ($p<0\ 05$) longer duration for side hole than for end hole catheters The difference is due to the fact that more infants died in the end hole catheter groups 11 in all Most of these infants died early often during the first day

The children with and without thrombosis were compared with regard to several parameters (Table 5) There were no significant differences for gestational age birth weight Apgar score degree of illness duration of catheterization until angiography or the infusion of bicarbonate Including the infants who died there was significantly ($p<0\ 01$)

Table 4 *Catheter type and clinical data comparison between the four groups*

Number of infants within parenthesis refer to duration of catheterization until angiography Mean ± S D

Type and position of catheter	No of infants	Birth weight (g)	Gestational age (weeks)	Apgar score (5 min)	Degree of illness	Duration of catheterization until angiography (hours)	No of infants receiving Sodium bicarbonate	No of infants receiving Blood transfusion
Long end hol	0 (14)	8 ±90?	35 8±3 1	7 ?±? 5	2 9±1 5	61± 7	8	3
Short end hole	19 (15)	? 060±75?	33 7±3 7	8 0±? 0	2 8±1 3	58± 3	11	4
Long side hole	1? (11)	2 090±925	34 ?±3 ?	8 0±1 8	6±1 0	64±44	6	3
Short side hole	11 (11)	? 046±701	34 1±4 5	7 ?± 3	- 6±0 5	88±47	6	3

Table 1 *Previous investigations in which the frequency of thrombosis at umbilical artery catheterization has been examined by angiography and autopsy*

Author	Type and position of catheter	Angiography n	Thrombosis (%)	Method	Autopsy n	Thrombosis (%)
Neal et al 1972 (20)	Long end hole	19	95	Cine	12	58
Strauss et al 1974 (24)	Short end hole	24	90	Cine		
Olinsky et al (1975 (21)	Long and short end hole	30	30	Several pictures	8	63
Goetzman et al 1975 (8)	Long end hole	98	24	One picture	42	21
Mokrohisky et al 1978 (19)	Long and short end hole	23	91	One picture		

MATERIAL

An attempt to catheterize an umbilical artery was performed on children fulfilling one or more of the clinical criteria given in Table 2 The catheterization was successful in 71 of 88 children (81%) The infants were randomized into 4 groups according to catheter type and localisation of the tip Long end hole (Th_{6-11}) (LE) short end hole (L_{3-4}) (SE) long side hole (LS) and short side hole (SS) catheters

These four groups were studied in parallel during a 15 months period During the next four months the infants were separated in two groups only short and long end hole catheters

There were 9 drop-outs among the 71 children (Table 3) 4 because it was not possible to evaluate the angiography 3 due to an occlusion of the catheter without indication for recatheterization and 2 for other reasons The remaining 62 infants are also shown in Table 3 Thirteen of these died none of them because of catheter complications There were no statistically significant differences between the four groups with regard to gestational age birth weight Apgar score duration of catheterization until angiography bicarbonate infusion blood transfusion through the catheter and degree of illness (Table 4) Degree of illness was estimated according to a 5 degree scale where score 5 consisted of the infants who died score 4 and 3 the infants who needed mechanical ventilation and continuous positive pressure (CPAP) respectively and score 2 and 1 the remaining less sick infants Four infants received total parenteral nutrition (Intralipid® and Vamine®) via the umbilical artery catheter

Table 2 *Indications for umbilical artery catheterization*

1 Birth weight below 1800 g
2 Gestational age 34 weeks or less
3 Postnatal asphyxia Apgar score below 7 at 5 min or no spontaneous breathing after 5 min
4 Breathing difficulties Silverman score more than 3 at 1 hour s age or clinical deterioration
5 Pronounced intrauterine growth retardation
6 Other severe neonatal diseases

METHODS

The catheterizations were done under strict aseptic conditions with a technique comparable to that recommended by Kitterman et al (13) Argyle umbilical artery catheter with end hole or Argyle feeding tube with side hole both made of polyvinyl chloride (PVC) were used French size no 5 was the common size except in 5 infants where French size 3 5 was used A high position of the catheter tip (Th_{6-11}) was reached by introducing the catheter to a third of the child s body length (30) and a low position (L_{3-4}) by using the body length and the figures given by Dunn (6) The position of the catheter was controlled by X ray and adjusted if necessary A 10% glucose solution was infused through the catheter If necessary blood transfusion or other solutions including sodium bicarbonate were given through the catheter The catheter was flushed with saline when withdrawing a blood sample or at least every 6th hour A new catheter was inserted if it was no longer possible to withdraw a blood sample and there was still a need for blood samples Heparin was not given The catheter was withdrawn as soon as the clinical condition permitted Before withdrawal angiography was performed by injecting manually 2–5 ml 45% Urografin® through the indwelling catheter A cine film recording was performed in the first 33 infants but there after one picture angiography was used Thrombosis embolism was considered to be present when at angiography an occlusion of the great pelvic or leg arteries was seen when filling defects in the aorta or pelvic arteries were seen or when there was an obvious sheath around the catheter

At autopsy the aorta and all its major branches were dissected The catheter was left in situ until autopsy

The function of the catheter at aspiration and infusion

Table 3 *The occurrence of thrombosis in relation to type and position of the umbilical artery catheter*

In infants both angiography and autopsy were performed

Type and position of catheter	Infants (n)	Infants with angiography (n)	Thrombosis at angiography (n)	Infants with autopsy (n)	Thrombosis at autopsy (n)	Total number of thrombosis n	Total number of thrombosis %	Drop outs (n)
Long end hole	20	14	0	6	0	0	0	2
Short end hole	19	15	3	5	2	5	26 3	4
Long side hole	12	11	4	1	0	4	33 3	1
Short side hole	11	11	7	1	0	7	63 6	2
Total	6	51	14	13	2	16	25 8	9

was registered as well as clinical signs that could be attributed to the catheterization

The investigation was approved by the ethical committee of the medical faculty of the University of Linkoping

RESULTS

The distribution of the 62 infants as well as the number of thromboses within the four groups s shown in Table 3 Sixteen infants (26%) had hrombosis or embolus Fourteen were diag iosed at angiography and 2 at autopsy Throm osis was present in all sub groups except LE atheter The difference in number of throm oses between long and short catheters (4 and 12) was significant ($p<0$ 05) as well as the difference between end and side hole catheters (5 and 11) ($p<0$ 01) There was also a significant difference between LE and LS catheters ($p<0$ 05) and between LE and SE catheters ($p<0$ 05) The most pronounced difference ($p<0$ 001) was found between LE and SS catheters There was some difference though not significant between these four groups in the duration of catheterization as calculated from the introduction of the catheter until angiography (Table 4) Including the infants who died in whom the duration of catheterization was calculated until the moment the infant died there was significantly ($p<0$ 05) longer duration for side hole than for end hole catheters The difference is due to the fact that more infants died in the end hole catheter groups 11 in all Most of these infants died early often during the first day

The children with and without thrombosis were compared with regard to several parameters (Table 5) There were no significant differences for gestational age birth weight Apgar score degree of illness duration of catheterization until angiography or the infusion of bicarbonate Including the infants who died there was significantly ($p<0$ 01)

Table 4 *Catheter type and clinical data comparison between the four groups*

Number of infants within parenthesis refer to duration of catheterization until angiography Mean ± S D

Type and position of catheter	No of infants	Birth weight (g)	Gestational age (weeks)	Apgar score (5 min)	Degree of illness	Duration of catheterization until angiography (hours)	No of infants receiving Sodium bicarbonate	No of infants receiving Blood transfusion
Long end hole	0 (14)	2 ?8?±90?	35 8±3 1	7 ±2 5	? 9±1 5	61± 7	8	3
Short end hole	19 (15)	? 060±75?	33 7±3 7	8 0±? 0	? 8±1 3	58± 3	11	4
Long side hole	1 (11)	090±9?5	34 ?±3 ?	8 0±1 8	? 6±1 0	64±44	6	3
Short side hole	11 (11)	? 046±701	34 1±4 5	7 ?± 3	? 6±0 5	88±47	6	3

Table 5 *Comparison between infants with and without thrombosis with regard to clinical data*
Number of infants within parenthesis refers to duration of catheterization until angiography Mean ± S D

	No of infants	Birth weight (g)	Gestational age (weeks)	Apgar score (5 min)	Degree of illness	Duration of catheterization until angiography (hours)	No of infants receiving: Sodium bicarbonate	Blood transfusion
Infants without thrombosis	46 (37)	2 096±891	34 2±3 7	7 5±2 2	2 7±1 3	61±32	20	7
Infants with thrombosis	16 (14)	2 249±555	35 6±3 1	7 9±2 2	2 9±1 0	82±43	11	6

longer duration of catheterization for the infants with than without thrombosis

Blood transfusion were more often given to children with thrombosis than without 38 and 15% respectively *Three of the 4 infants who* received total parenteral nutrition via the umbilical catheter had thrombosis Exclusion of these 4 infants from the total material does not change the significant differences between the 4 catheter groups There was no significant difference between the groups with regard to haemoglobin haematocrit activated partial thromboplastin time (APTT) or number of platelets

No case of septicemia was found but blood and catheter cultures were positive in 13 and 28% respectively The infants who developed thrombosis did not show an increased number of positive cultures For further details regarding these aspects see Wesstrom et al (31)

Table 6 *Catheter problems at aspiration or infusion and recatheterization*

Type and position of catheter	No of infants	No of infants with catheter problems	No of infants recatheterized
Long end hole	22	2	–
Short end hole	23	13	5
Long side hole	13	3	2
Short side hole	13	11	6
Total	71	29	13

Three of the infants with thrombosis had a complete occlusion of the common iliac artery on the side of the catheterization All these 3 infants had SS catheters One infant with a SE *catheter had an embolus* which totally occluded the popliteal artery on the side opposite to that of the catheterization

Colour changes of the legs were noted in 18 infants The duration of these was short as a rule Six of these infants had thrombosis or embolism Thus ten infants with thrombosis lacked such signs

The function of the catheters at aspiration and infusion was investigated There were difficulties mainly with the short catheters and particularly with the SS catheters (Table 6) In three infants with SE catheters the catheter slipped out spontaneously in spite of fixation with a pursestring suture There was no bleeding For 69% of the infants with thrombosis there were problems with the catheter The corresponding figure for infants without thrombosis was 28%

DISCUSSION

The use of umbilical artery catheters in the care of sick newborn infants is a widely accepted method for getting samples for blood gas analysis Other methods for receiving arterial blood such as repeated puncture or catheterization of the temporal or radial artery have limitations (1 4 27 29) Transcutaneous P_{O_2} monitoring will probably reduce but not

replace the need of umbilical artery catheters in the future (12 18 25)

Umbilical artery catheterization involves certain risks The most serious complication has been thrombosis or embolism The frequency has varied considerably from 3 5 to 48% in postmortem investigations (5 9 16 33) and from 24 to 95% in earlier angiographic investigations (Table 1) Different types of catheters and positions of the tips have been used In the Swedish inquiry it was found that all four catheter types and positions that we studied were in use (7)

The present investigation showed a frequency of thromboses of 26% The result is difficult to compare with previous investigations as the frequency varies with the type and position of the catheter (Table 3) Neal et al (20) as well as Goetzman et al (8) used LE catheters and found a frequency of thromboses of 95 and 24% respectively to be compared with 0% for the same type and position of the catheter in our study Strauss et al (24) used SE catheters and found thrombosis in 90% compared to 26% in the present investigation and 30% in the mixed (LE + SE) catheter material of Olinsky et al (21) The great discrepancy between these results and ours is difficult to explain Olinsky et al (21) showed that a catheter filled with contrast medium and X rayed in a contrast solution shows a translucence around the catheter Translucence can easily be mistaken for thrombosis This finding might explain some very high figures for thrombosis given in early investigations

A possible advantage of side hole catheters is the smooth tip which theoretically may lessen the risk for damage of the vessel intima and thus for thrombosis The present study showed quite the contrary A reason might be the dead space in the tip of the side hole catheter where coagulation might occur leading to thrombosis

An explanation for the difference in thrombosis between long and short catheters is more [illegible] ther study we failed to find a difference in fibrinolytic activity between different parts of the aorta (11) Another explanation could be a difference in blood velocity between the thoracic and the abdominal aorta The blood flow in the thoracic aorta is greater than in the abdominal aorta since a great part of the blood leaves for the internal organs The angiographic films showed that the abdominal aorta was only slightly smaller than the thoracic aorta Thus the blood velocity in the abdominal aorta is probably slower which might lead to an increased risk for developing thrombosis

Total parenteral nutrition via the umbilical artery catheter probably increases the risk for developing thrombosis since 3 of 4 who received parenteral nutrition had thrombosis

A long duration of the catheterizations has been said to enhance the risk for thrombosis (26) Goetzman et al (8) performed repeated angiographies on some infants and showed that when the first examination was negative the others were negative as well In the present study there was a difference in the duration of the catheterization between infants with and without thrombosis Therefore we think that the duration of the catheterization is at least of some importance for the occurrence of thrombosis

Materials other than PVC in the catheters have been tried Boros et al (3) compared PVC and Silicone Elastomere (Silastic) catheters in a study of 20 infants They found 90 and 10% thromboses respectively in the two non randomized groups The Silastic catheter is considerably softer and probably more difficult to handle and when used by the same authors in animal experiments thromboses were found in all cases at autopsy

The present investigation as well as several earlier studies (9 16 20) have shown a poor correlation between clinical signs and thrombosis As a rule therefore it is not possible to diagnose thrombosis from clinical signs only

The function of the catheters is of great importance A poorly functioning catheter

often has to be exchanged which may enhance the risk of complications As shown in the results (Table 6) the function of the catheters varied considerably with different positions

Angiography through the indwelling catheter is a safe method for diagnosing thrombosis in the aorta and its major branches No complications related to the angiography were noted in this study nor in earlier reports In the first part of this study cine angiography was used later on replaced by one picture angiography The reason for this change was that the technically successful one picture angiography gave the same degree of information with a lower radiation dose and in a more simple way The diagnosis of thrombosis by angiography may be difficult and under and overestimation of thrombosis may occur In order to overcome this problem two experienced roentgenologists evaluated all doubtful films in the present study In 12 of 14 cases (Table 3) the diagnosis of thrombosis was regarded as without doubt In the remaining 2 cases the diagnosis was probable In one of them there were only sleeve like changes around the tip of the catheter and in the other there was a difference in blood pressure between the two legs a finding that we have seen in another study (32) to be related to arterial thrombosis Therefore the diagnosis based on angiography was probably correct

Most authors recommend a low position of the catheter tip (13 20 21 28) The present investigation showed however that the risk of thrombosis is significantly lower with a high position in which the catheters also function better In two recent papers clinical signs of complications have been related to the position of the catheter tip (10 19) Both groups arrived at the same conclusion as we did i e a higher complication rate with a low catheter position Thrombosis occurring with a high position of the catheter tip might theoretically lead to more serious consequences as the thrombosis may then affect the mesenteric or renal arteries (20 21 28) This is the main reason why most authors recommend a low catheter position with the tip below the level of the main vessels to the internal organs However these main vessels can be affected by thrombosis even when a short catheter is used perhaps from retrograde movement of clots (19) Furthermore a satisfactory low position is not always easy to obtain not even with fluoroscopy as the level at which the main vessels take off from the aorta varies (21 22 30) When using a high position of the catheter such problems do not exist

In conclusion we believe that there is substantial evidence to recommend end hole catheters in a high position (Th_{6-11}) when performing umbilical artery catheterization in the newborn infant

ACKNOWLEDGEMENT

This work was supported by grants from Prenatal forskningsfonden

REFERENCES

1 Adams J M & Rudolph A J The use of indwelling radial artery catheters in neonates *Pediatrics* 55 261 1975
2 Andersson A Hofer P Å Holmlund D E W & Wendel H Å Colonic perforation secondary to thrombo-embolus from an umbilical artery catheter *Acta Paediatr Scand* 63 155 1974
3 Boros S J Thomson T R Reynolds J W Jarvis C W & Williams H J Reduced thrombus formations with silicone elastomere (silastic) umbilical artery catheters *Pediatrics* 56 981 1975
4 Bucci G Scalamandre A Savignoni P G Orzalesi M & Mendicini M Crib-side sampling of blood from the radial artery *Pediatrics* 37 497 1966
5 Cochran W D Davis H T & Smith C A Advantages and complications of umbilical artery catheterization in the newborn *Pediatrics* 42 769 1968
6 Dunn P M Localization of the umbilical catheter by postmortem measurement *Arch Dis Child* 41 69 1966
7 Finnstrom O Stenport G & Wesstrom G Catheterization of the umbilical artery in neonates *Lakartidningen* 75 2627 1978
8 Goetzman B W Stadalnik R C Bogren H G Blankenship W J Ikeda R M & Thayer J Thrombotic complications of umbilical artery catheters A clinical and radiographic study *Pediatrics* 56 374 1975
9 Gupta J M Roberton N R C & Wigglesworth J S Umbilical artery catheterization in the newborn *Arch Dis Child* 43 382 1968

10 Harris M S & Little G A Umbilical artery catheters High low or no J Perinat Med 6 15 1978
11 Henriksson P Wesstrom G & Hedner U Umbilical artery catheterization in newborns III Thrombosis—A study of some predisposing factors Acta Paed atr Scand in press
1? Huch R Lubbers D W & Huch A Reliability of transcutaneous monitoring of arterial PO in newborn infants Arch Dis Child 49 ?13 1974
13 Kitterman J A Phibbs R H & Tooley W H Catheterization of umbilical vessels in newborn infants Pediatr Clin North Am 17 895 1970
14 Koch G & Wendel H Comparison of Ph carbon dioxide tension standard bicarbonate and oxygen tension in capillary blood and in arterial blood during the neonatal period Acta Paediatr Scand 56 10 1967
15 Krauss A N Albert R F & Kannan M M Contamination of umbilical catheters in the newborn infant J Pediatr 77 965 1970
16 Larroche J Cl Umbilical catheterization Its complications Biol Neonate 16 101 1970
17 Livaditis A Wallgren G & Faxelius G Necrotizing enterocolitis after catheterization of the umbilical vessels Acta Paediatr Scand 63 ?77 1974
18 Lofgren O Henriksson P Jacobson L & Johansson O Transcutaneous PO monitoring in neonatal intensive care Acta Paediatr Scand 67 693 1978
19 Mokrohisky S T Levine R L Blumhagen J D Wesenberg R L & Simmons M A Low positioning of umbilical artery catheters increases associated complications in newborn infants N Engl J Med 299 561 1978
?0 Neal W A Reynolds J W Jarvis C W & Williams H J Umbilical artery catheterization Demonstration of arterial thrombosis by aortography Ped atrics 50 6 197
?1 Olinsky A Aitken F G & Isdale J M Thrombus formation after umbilical arterial catheterisation An angiographic study S Afr Med J 49 1467 1975
?? Phelps D L Lachman R S Leake R D & Oh W The radiologic localization of the major aortic tributaries in the newborn infant J Pediatr 81 336 1972
23 Rajs J Finnstrom O & Wesstrom G Aortic aneurysm developing after umbilical artery catheterization Acta Paediatr Scand 65 495 1976
?4 Strauss A W Escobedo M & Goldring D Continuous monitoring of arterial oxygen tension in the newborn infant J Pediatr 85 254 1974
?5 Swanstrom S & Rooth G The value of transcutaneous PO_2 measurements in a newborn infant in shock Paediatrician 5 304 1976
?6 Symansky M R & Fox H A Umbilical vessel catheterization Indications management and evaluation of the technique J Pediatr 80 8?0 1972
27 Thomsen A Arterial blood sampling in small infants Acta Paediatr Scand 53 237 1964
?8 Tooley W H What is the risk of an umbilical artery catheter? Pediatrics 50 1 197?
?9 Wall P M & Kuhns L R Percutaneous arterial sampling using transillumination Pediatrics 59 103? 1977
30 Weaver R L & Ahlgren E W Umbilical artery catheterization in neonates Am J Dis Child 1?2 499 1971
31 Wesstrom G & Finnstrom O Umbilical artery catheterization in newborns II Infections in relation to catheterization Acta Paed atr Scand in press
32 Wesstrom G & Lassvik C To be published
33 Wigger H J Bransilver B R & Blanc W A Thrombosis due to catheterization in infants and children J Pediatr 76 1 1970

Submitted Oct ?6 1978
Accepted Jan 5 1979

(G W) Department of Paediatrics
University Hospital
S 581 85 Linkoping
Sweden

often has to be exchanged which may enhance the risk of complications As shown in the results (Table 6) the function of the catheters varied considerably with different positions

Angiography through the indwelling catheter is a safe method for diagnosing thrombosis in the aorta and its major branches No complications related to the angiography were noted in this study nor in earlier reports In the first part of this study cine angiography was used later on replaced by one picture angiography The reason for this change was that the technically successful one picture angiography gave the same degree of information with a lower radiation dose and in a more simple way The diagnosis of thrombosis by angiography may be difficult and under and overestimation of thrombosis may occur In order to overcome this problem two experienced roentgenologists evaluated all doubtful films in the present study In 12 of 14 cases (Table 3) the diagnosis of thrombosis was regarded as without doubt In the remaining 2 cases the diagnosis was probable In one of them there were only sleeve like changes around the tip of the catheter and in the other there was a difference in blood pressure between the two legs a finding that we have seen in another study (32) to be related to arterial thrombosis Therefore the diagnosis based on angiography was probably correct

Most authors recommend a low position of the catheter tip (13 20 21 28) The present investigation showed however that the risk of thrombosis is significantly lower with a high position in which the catheters also function better In two recent papers clinical signs of complications have been related to the position of the catheter tip (10 19) Both groups arrived at the same conclusion as we did i e a higher complication rate with a low catheter position Thrombosis occurring with a high position of the catheter tip might theoretically lead to more serious consequences as the thrombosis may then affect the mesenteric or renal arteries (20 21 28) This is the main reason why most authors recommend a low catheter position with the tip below the level of the main vessels to the internal organs However these main vessels can be affected by thrombosis even when a short catheter is used perhaps from retrograde movement of clots (19) Furthermore a satisfactory low position is not always easy to obtain not even with fluoroscopy as the level at which the main vessels take off from the aorta varies (21 22 30) When using a high position of the catheter such problems do not exist

In conclusion we believe that there is substantial evidence to recommend end hole catheters in a high position (Th_{6-11}) when performing umbilical artery catheterization in the newborn infant

ACKNOWLEDGEMENT

This work was supported by grants from Prenatal forskningsfonden

REFERENCES

1 Adams J M & Rudolph A J The use of indwelling radial artery catheters in neonates *Pediatrics 55* 261 1975
2 Andersson A Hofer P Å Holmlund D E W & Wendel H Å Colonic perforation secondary to thrombo embolus from an umbilical artery catheter *Acta Paediatr Scand 63* 155 1974
3 Boros S J Thomson T R Reynolds J W Jarvis C W & Williams H J Reduced thrombus formations with silicone elastomere (silastic) umbilical artery catheters *Pediatrics 56* 981 1975
4 Bucci G Scalamandre A Savignoni P G Orzalesi M & Mendicini M Crib-side sampling of blood from the radial artery *Pediatrics 37* 497 1966
5 Cochran W D Davis H T & Smith C A Advantages and complications of umbilical artery catheterization in the newborn *Pediatrics 42* 769 1968
6 Dunn P M Localization of the umbilical catheter by postmortem measurement *Arch Dis Child 41* 69 1966
7 Finnstrom O Stenport G & Wesstrom G Catheterization of the umbilical artery in neonates *Lakartidningen 75* 2627 1978
8 Goetzman B W Stadalnik R C Bogren H G Blankenship W J Ikeda R M & Thayer J Thrombotic complications of umbilical artery catheters A clinical and radiographic study *Pediatrics 56* 374 1975
9 Gupta J M Roberton N R C & Wigglesworth J S Umbilical artery catheterization in the newborn *Arch Dis Child 43* 382 1968

Acta Pædiatr Scand 68 583–585 1979

ADYNAMIA EPISODICA HEREDITARIA

Treatment with Salbutamol

K DAHL JØRGENSEN and H MICHALSEN

From the Department of Paediatrics Aker Hospital Oslo Norway

ABSTRACT Dahl Jørgensen K and Michalsen H (Department of Paediatrics Aker Hospital Oslo Norway) Adynamia episodica hereditaria Treatment with salbutamol Acta Paediatr Scand 68 583 1979 —Three sibs with adynamia episodica hereditaria are described Treatment with salbutamol inhalation had a beneficial effect on the duration of their adynamic attacks Continuous peroral treatment with salbutamol has been tried in these patients resulting in the almost complete prevention of attacks No serious adverse effects have been recorded Peroral treatment with salbutamol is recommended as the treatment of choice in young patients with adynamia episodica hereditaria and in patients with frequent attacks of adynamia

KEY WORDS Adynamia episodica hereditaria salbutamol treatment

Adynamia episodica hereditaria (AEH) a disease inherited as an autosomal dominant is characterized by early onset of short and frequent episodes of muscle weakness accompanied by increasing serum potassium (1) Drugs which are reported to shorten an attack when given during an attack are calcium intravenously catecholamines subcutaneously and inhalation of salbutamol (2 3 4) The effect of salbutamol has in vitro been shown to be related to the hypokalemic effect due to stimulation of the active Na and K transport in the muscle cells (4) Acetazolamid has for many years been used successfully to prevent attacks (1 2)

The diagnosis of AEH has been established in 3 siblings their mother being the only previously known case of AEH in Norway (2) The beneficial effect of salbutamol inhalation was confirmed in these patients Prophylactic treatment with salbutamol successfully reduced the frequency of the attacks

PATIENTS

The mother of our patients had early manifestations of AEH but her diagnosis was not confirmed until 10 years ago The father of our patients has no sign of AEH The couple have 3 children all suffering from AEH

Patient 1 is a girl (13 years old) patients 2 and 3 are boys (9 and 7 years old) Their symptoms started before 1 year of age Their mother describes the symptoms as short periods of muscular hypotonia At the age of 12 14 months the children commenced to walk and from then on the muscular weakness has appeared in rather distinct attacks Intellectual and motor development is normal Patient 2 had an attack of supraventricular paroxysmal tachycardia 7 years old the children have otherwise not suffered from serious diseases No previous examinations have been performed

The pattern of attacks is identical in the 3 patients They have from 2 to 4 daily attacks each attack lasting 15–30 min During these episodes the children are unable to walk or maintain an upright position Nightly attacks occur on the average once a week in each patient These are more severe generally lasting for 2–3 hours and are regularly accompanied by the subjective experience of respiratory distress The parents confirm that during nightly attacks the respiration appears distressed audible respirations are present with both inspiratory and expiratory stridor Patient 1 has moderate signs of myotonia in addition to the attacks of adynamia No treatment has been tried prior to their admission in our department but the children had experienced that active or passive movements of the affected limbs did shorten the attacks

RESULTS

Clinical and neurological examination of the children was normal when performed in intervals between the attacks

Acta Pædiatr Scand 68 583–585 1979

ADYNAMIA EPISODICA HEREDITARIA

Treatment with Salbutamol

K DAHL JORGENSEN and H MICHALSEN

From the Department of Paediatrics Aker Hospital Oslo Norway

ABSTRACT Dahl Jørgensen K and Michalsen H (Department of Paediatrics Aker Hospital Oslo Norway) Adynamia episodica hereditaria Treatment with salbutamol Acta Paediatr Scand 68 583 1979 —Three sibs with adynamia episodica hereditaria are described Treatment with salbutamol inhalation had a beneficial effect on the duration of their adynamic attacks Continuous peroral treatment with salbutamol has been tried in these patients resulting in the almost complete prevention of attacks No serious adverse effects have been recorded Peroral treatment with salbutamol is recommended as the treatment of choice in young patients with adynamia episodica hereditaria and in patients with frequent attacks of adynamia

KEY WORDS Adynamia episodica hereditaria salbutamol treatment

Adynamia episodica hereditaria (AEH) a disease inherited as an autosomal dominant is characterized by early onset of short and frequent episodes of muscle weakness accompanied by increasing serum potassium (1) Drugs which are reported to shorten an attack when given during an attack are calcium intravenously catecholamines subcutaneously and inhalation of salbutamol (2 3 4) The effect of salbutamol has in vitro been shown to be related to the hypokalemic effect due to stimulation of the active Na and K transport in the muscle cells (4) Acetazolamid has for many years been used successfully to prevent attacks (1 2)

The diagnosis of AEH has been established in 3 siblings their mother being the only previously known case of AEH in Norway (2) The beneficial effect of salbutamol inhalation was confirmed in these patients Prophylactic treatment with salbutamol successfully reduced the frequency of the attacks

PATIENTS

The mother of our patients had early manifestations of AEH but her diagnosis was not confirmed until 10 years ago The father of our patients has no sign of AEH The couple have 3 children all suffering from AEH

Patient 1 is a girl (13 years old) patients 2 and 3 are boys (9 and 7 years old) Their symptoms started before 1 year of age Their mother describes the symptoms as short periods of muscular hypotonia At the age of 12–14 months the children commenced to walk and from then on the muscular weakness has appeared in rather distinct attacks Intellectual and motor development is normal Patient had an attack of supraventricular paroxysmal tachycardia 7 years old the children have otherwise not suffered from serious diseases No previous examinations have been performed

The pattern of attacks is identical in the 3 patients They have from ? to 4 daily attacks each attack lasting 15–30 min During these episodes the children are unable to walk or maintain an upright position Nightly attacks occur on the average once a week in each patient These are more severe generally lasting for 2–3 hours and are regularly accompanied by the subjective experience of respiratory distress The parents confirm that during nightly attacks the respiration appears distressed audible respirations are present with both inspiratory and expiratory stridor Patient 1 has moderate signs of myotonia in addition to the attacks of adynamia No treatment has been tried prior to their admission in our department but the children had experienced that active or passive movements of the affected limbs did shorten the attacks

RESULTS

Clinical and neurological examination of the children was normal when performed in intervals between the attacks

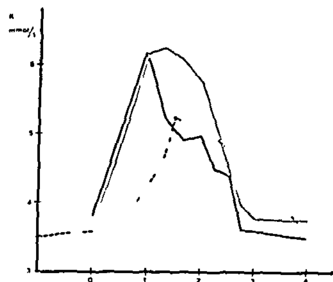


Fig. 1. Serum concentrations of potassium in the three patients during an attack.

Table I. *Number of adynamic attacks during 1 week without treatment (Period I), during 1 week with placebo (Per. II), and during 1 week with salbutamol. Periods II and III were performed double blind, the tablets having identical appearance*

	Period I	Period II	Period III
Patient 1	14	13	[illegible]
Patient 2	14	14	7
Patient 3	11	12	1

Muscular weakness occurred spontaneously in patient 1 and 2. In patient 3 an episode was provoked during rest following 30 min of muscular exercise. During the attacks of adynamia we found flaccid weakness in the muscles of the face and extremities, the weakness being most pronounced in the lower limbs. The tendon reflexes were weaker than prior to the attack.

Elevated serum levels of potassium (Fig. 1) as well as hyperkalemic ECG changes were observed in all 3 patients during the attack. Electromyography performed between attacks is described as normal, the only possible abnormality being a moderate increase in spontaneous activity. Peripheral motor conduction rates were within normal limits. Assays of T_3, T_4 and TSH did not reveal any signs of thyroid disorder. In patients 1 and 3 biopsies from the anterior tibial muscle were performed. Light microscopy and electron microscopy revealed no distinct abnormalities.

Inhalation of salbutamol alleviated hyperkalemia and clinical symptoms within few minutes. Patient 1 managed to use the inhalator according to instructions and was satisfied with the effect of this treatment. The two younger patients tried the inhalator during a later phase of the attacks and were therefore often unable to manage it effectively due to weakness of the upper limbs. However, the duration of their attacks were shortened by inhalations when they were helped by others. In some cases the inhalations had to be repeated once or twice in order to alleviate the symptoms.

We determined to try salbutamol tablets as a possible prophylactic treatment. Salbutamol was administered in doses of 0.3–0.4 mg/kg body weight divided in 4 daily doses. On this treatment the patients have been almost free from the episodes of adynamia. Patient 1 has continued, however, to have a moderate increase in the frequency of the myotonic episodes. No side effects have been noticed.

After more than 6 months on peroral salbutamol treatment we decided to perform a trial with placebo tablets having identical taste and appearance as the salbutamol tablets. The patients were without treatment for one week prior to the trial, each attack during this and the following weeks was carefully recorded by their mother. Placebo and salbutamol were given on alternating weeks; neither their mother nor their pediatrician knew which of the tablets the patients were given. The results of this trial are summarized in Table I. The beneficial effect of prophylactic treatment is clearly demonstrated.

DISCUSSION

The diagnosis of these 3 patients is based on the typical history and the elevated serum

concentrations of potassium during the attacks of muscle weakness

Treatment with salbutamol inhalations was of questionable value in our patients due to the frequency and severity of their attacks The two younger patients had trouble learning the technique of correct and early inhalation After the initiation of peroral treatment the children have been virtually free from attacks The observation period is presently 9 months Trial with placebo tablets resulted in prompt recurrence of the attacks

The doses of salbutamol presently used have had no detectable symphatomimetic effects We are not aware of serious adverse reactions related to long term treatment with salbutamol The patients do however demonstrate more myotonic attacks than prior to salbutamol therapy this effect is most pronounced in the oldest patient During the placebo experiment her myotonia almost disappeared she did however have two daily attacks of adynamia during this period

Acetazolamid has until now been the drug of choice in the prevention of weakness in AEH This drug is known to have numerous though rare side effects skin reactions liver damage bone marrow depression kidney lesions resembling those seen with sulphonamides and renal calculi are among the adverse reactions related to treatment with acetazolamid Long term treatment with acetazolamid may thus be more risky than with salbutamol Our patients have responded well to this apparently safe drug We therefore recommend the use of peroral salbutamol treatment in young patients with AEH and in patients with frequent attacks

REFERENCES

1 Gamstorp I Adynamia episodica hereditaria *Acta Paediatr Scand 45* Suppl 108 1956
2 Hovstad L Henriksen O & Try K Adynamia episodica hereditaria *Nord Med 80* 1065 1968
3 Klein R Egan T & Usher P Changes in sodium potassium and water in hyperkalemic familial periodic paralysis *Metabolism* 9 1005 1960
4 Wang P & Clausen T Treatment of attacks in hyperkalæmic familial periodic paralysis by inhalation of salbutamol *Lancet I* 2 1 1976

Submitted Dec 4 1978
Accepted Jan 25 1979

(H M) Department of Paediatrics
Aker Hospital
Oslo 5
Norway

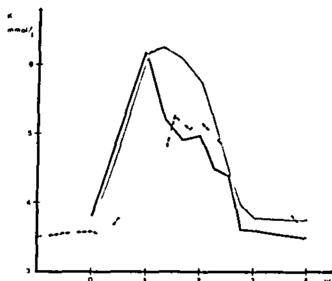


Fig. 1. Serum concentrations of potassium in the three patients during an attack.

Muscular weakness occurred spontaneously in patient 1 and 2. In patient 3 an episode was provoked during rest following 30 min of muscular exercise. During the attacks of adynamia we found flaccid weakness in the muscles of the face and extremities, the weakness being most pronounced in the lower limbs. The tendon reflexes were weaker than prior to the attack.

Elevated serum levels of potassium (Fig. 1) as well as hyperkalemic ECG changes were observed in all 3 patients during the attack. Electromyography performed between attacks is described as normal, the only possible abnormality being a moderate increase in spontaneous activity. Peripheral motor conduction rates were within normal limits. Assays of T_3, T_4 and TSH did not reveal any signs of thyroid disorder. In patients 1 and 3 biopsies from the anterior tibial muscle were performed. Light microscopy and electron microscopy revealed no distinct abnormalities.

Inhalation of salbutamol alleviated hyperkalemia and clinical symptoms within few minutes. Patient 1 managed to use the inhalator according to instructions and was satisfied with the effect of this treatment. The two younger patients tried the inhalator during a later phase of the attacks and were therefore often unable to manage it effectively due to weakness of the upper limbs. However the duration of their attacks were shortened by inhalations when they were helped by others. In some cases the inhalations had to be repeated once or twice in order to alleviate the symptoms.

We determined to try salbutamol tablets as a possible prophylactic treatment. Salbutamol was administered in doses of 0.3–0.4 mg/kg body weight divided in 4 daily doses. On this treatment the patients have been almost free from the episodes of adynamia. Patient 1 has continued, however, to have a moderate increase in the frequency of the myotonic episodes. No side effects have been noticed.

After more than 6 months on peroral salbutamol treatment we decided to perform a trial with placebo tablets having identical taste and appearance as the salbutamol tablets. The patients were without treatment for one week prior to the trial; each attack during this and the following weeks was carefully recorded by their mother. Placebo and salbutamol were given on alternating weeks; neither their mother nor their pediatrician knew which of the tablets the patients were given. The results of this trial are summarized in Table 1. The beneficial effect of prophylactic treatment is clearly demonstrated.

Table 1. *Number of adynamic attacks during 1 week without treatment (Period I), during 1 week with placebo (Per. II) and during 1 week with salbutamol. Periods II and III were performed double blind, the tablets having identical appearance*

	Period I	Period II	Period III
Patient 1	14	13	[illegible]
Patient 2	14	14	7
Patient 3	11	12	1

DISCUSSION

The diagnosis of these 3 patients is based on the typical history and the elevated serum

concentrations of potassium during the attacks of muscle weakness

Treatment with salbutamol inhalations was of questionable value in our patients due to the frequency and severity of their attacks The two younger patients had trouble learning the technique of correct and early inhalation After the initiation of peroral treatment the children have been virtually free from attacks The observation period is presently 9 months Trial with placebo tablets resulted in prompt recurrence of the attacks

The doses of salbutamol presently used have had no detectable symphatomimetic effects We are not aware of serious adverse reactions related to long term treatment with salbutamol The patients do however demonstrate more myotonic attacks than prior to salbutamol therapy this effect is most pronounced in the oldest patient During the placebo experiment her myotonia almost disappeared she did however have two daily attacks of adynamia during this period

Acetazolamid has until now been the drug of choice in the prevention of weakness in AEH This drug is known to have numerous though rare side effects skin reactions liver damage bone marrow depression kidney lesions resembling those seen with sulphonamides and renal calculi are among the adverse reactions related to treatment with acetazolamid Long term treatment with acetazolamid may thus be more risky than with salbutamol Our patients have responded well to this apparently safe drug We therefore recommend the use of peroral salbutamol treatment in young patients with AEH and in patients with frequent attacks

REFERENCES

1 Gamstorp I Adynamia episodica hereditaria *Acta Paediatr Scand 45* Suppl 108 1956
2 Hovstad L Henriksen O & Try K Adynamia episodica hereditaria *Nord Med 80* 1065 1968
3 Klein R Egan T & Usher P Changes in sodium potassium and water in hyperkalemic familial periodic paralysis *Metabolism 9* 1005 1960
4 Wang P & Clausen T Treatment of attacks in hyperkalæmic familial periodic paralysis by inhalation of salbutamol *Lancet I* 721 1976

Submitted Dec 4 1978
Accepted Jan 25 1979

(H M) Department of Paediatrics
Aker Hospital
Oslo 5
Norway

Acta Pædiatr Scand 68 587–592 1979

MALE HYPOSPADIAS 625 CASES ASSOCIATED MALFORMATIONS AND POSSIBLE ETIOLOGICAL FACTORS

J SVENSSON

F m the Depa tment of Paediatric Surgery Karolinska sjukhuset Stockholm S eden

ABSTRACT Svensson J (Department of Paediatric Surgery Karolinska sjukhuset Stockholm Sweden) Male hypospadias 625 cases Associated malformations and possible etiological factors Acta Paediatr Scand 68 587 1978 —Records from 625 patients with hypospadias have been reviewed with reference to associated malformations Cryptorchidism was found to be the most common associated malformation and was present in 6% of cases with hypospadias The highest incidence of cryptorchidism was found in the more severe forms of hypospadias Other concomitant genital malformations such as bifid scrotum hypoplasia of penis and remnants of the Mullerian system showed the same pattern The recorded incidence of bifid scrotum and hypoplasia of the penis was 4% and 8% respectively A high incidence of abnormalities in the lower urinary tract was found 50% of 84 patients investigated The hypothesis of hypospadias being a result of testicular hormone insufficiency is discussed

KEY WORDS Hypospadias cryptorchidism lower urinary tract

Hypospadias in one of the most common developmental malformations with an incidence in Sweden of one case per 350 male births (4) In most cases the etiology of the malformation is unknown

Several authors have found a hereditary element in hypospadias Sørensen (32) reports that about 10% of brothers of hypospadic patients were affected Chen et al (7) estimate the heritability according to Falconer (10) as 74 1% indicating a polygenic etiology in most cases and that hereditary factors represent 74 1% of the causation of the malformation The risk of recurrence within one generation has been estimated as approximately 6% (23)

Male pseudohermaphrodites might be defined as individuals with testicular gonads whose external genitalia have not developed into the normal male appearance In this broad sense all males with hypospadias are male pseudohermaphrodites although in the majority of cases the male sex of hypospadic patients is not questioned In the more severe forms of hypospadias doubts may be raised as to the sex role in which the patient should be reared

In a cytogenetic study of 80 cases of hypospadias Aarskog (1) found six patients with abnormal karyotypes Three with XX/XY two with XO/XY mosaicism and one patient with a normal female karyotype the latter was a case of congenital virilizing adrenal hyperplasia All six had severe hypospadias of penile (one) penoscrotal (four) and scrotal (one) type A high incidence of sex chromosome aberrations is found in patients with hypospadias and associated cryptorchidism (2 26)

Some cases of male pseudohermaphroditism with hypospadias have been ascribed to errors in the biosynthesis of androgens (37) and defects in the peripheral action of androgens on the target cells in the genital region The development of the external genitalia in the male is dependent on testosterone secreted

Table 3 *Number of recorded malformations in the lower urinary tract in different types of hypospadias found at voiding cystourethrography*

Type of hypospadias	No of patients examined	Vesico ureteral reflux	Urethral folds: Obstructive	Urethral folds: Non obstructive	Urethral recesses
Glandular	17	2	–	5	1
Distal penile	8	3	3	5	–
Mid penile	9	1	1		1
Proximal penile	6	–	2	1	1
Penoscrotal	16	–	–	7	3
Scrotal	4	–	–	–	3
Perineal	4	–	1	–	2
	84	6	7	20	11

scrotum in 22%. Farkas also found that the incidence of associated anomalies was highest in patients with severe forms of hypospadias.

The aim of this *retrospective study* was to review a series of patients with hypospadias and the presence of associated malformations mainly as a background for studies on the etiology of hypospadias and the late somatic and *psychological* sequelae of this genital abnormality.

CLINICAL MATERIAL

During the period 195 –197? 6?5 boys were admitted to the Department of Paediatric Surgery, Karolinska sjukhuset, for treatment of hypospadias. Excluded from this study are patients with insignificant lesions not considered to require admittance for *surgical correction*.

The different degrees of hypospadias included in the survey are listed in Table 1. Operations (meatotomy, correction of chordee and urethroplasty) were carried out by several surgeons using techniques described by Sir Denis Browne (5) or by Crawford (8). Surgical treatment was not yet completed in all patients at the end of 197?. The median ages for meatotomy, correction of chordee and *urethroplasty were 5, 3 9* and *5* years respectively.

Table 4 *Associated malformations recorded different types of hypospadias*

Type of malformation	No of patients with the malformation	Type of hypospadias
Ventricular septal defect	1	Glandular
Deafness	1	Distal penile
VOC anal atresia	2	Glandular Penoscrotal
Hydrocephalus	1	Glandular
Omphalocele	1	Glandular
Esophageal atresia	1	Mid penile
Anal atresia		Mid penile

ASSOCIATED MALFORMATIONS

All figures in this study are based on records from patients during a 21 year period. In this kind of retrospective study there is a certain risk of under-estimation of associated malformations since these may not always have been properly recorded.

Cryptorchidism was recorded in 36 patients (6%), *bilateral in* ?3 cases (4%). The scrotum was bifid in ? patients (4%). Hypoplasia of the penis, as judged *subjectively by the surgeon*, was noted in 50 cases (8%). Like cryptorchidism and bifid scrotum, hypoplasia of the penis was found *more often in the more* severe forms of hypospadias (Table).

The lower urinary tract was *examined by voiding* cystourethrography in 84 patients (1?%) and of these 50% had some form of abnormality (Table 3).

Vesicoureteric reflux was seen in 6 patients. Transverse fold formations in the anterior wall of the prostatic urethra just opposite to the verumontanum were observed in ?5 patients. Of these folds five were slightly *obstructive*. True obstructive posterior *urethral* valves were observed in two patients.

Recesses *of various sizes* in the posterior wall of the prostatic and bulbous urethra were visualized at voiding cystourethrography *in 11 patients*. It could not be determined whether these recesses represented a partially developed vagina or dilated ducts of the bulbo urethral glands (?). However, in one patient with scrotal hypospadias the pouch continued *directly into* a uterus. Biopsies from the gonads of this patient showed both testicular and ovarian tissue. The urethral *pouches* were common in the more severe cases of hypospadias (Table 3).

Table 1. *Total number of patients with hypospadias included in the study and the different types of operations performed*

n = number of patients

	Total number		Meatotomy only		Meatotomy plus other urethral surgery		Correction of chordee		Urethroplasty	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%
Glandular	219	35	95	43	33	15	56	26	84	38
Distal penile	195	31	23	12	67	34	100	51	158	81
Mid penile	93	15	1	1	18	19	58	62	86	92
Proximal penile	43	7	–	–	11	26	32	74	39	91
Penoscrotal	42	7	–	–	3	7	37	88	36	88
Scrotal	11	2	–	–	1	9	10	91	10	91
Perineal	22	3	–	–	2	9	21	95	21	95
	625		119 (19%)		135 (15%)		314 (50%)		434 (69%)	

from the fetal testis (20). Testosterone is metabolized in the target cells of the external genital region by an enzyme 5α reductase to 5α dihydrotestosterone (5α DHT), which is supposed to be the active intracellular androgen in this region (30, 35). 5α DHT is bound to a soluble cytoplasmic receptor of protein nature and the 5α DHT protein complex is then transferred into the nucleus where it exerts its effects on the genome (24). A defective 5α reduction has been described in cases of pseudovaginal perineoscrotal hypospadias, a rare hereditary disease (33). Lack of androgen receptor as a cause of hypospadias in human males has also been described (14).

Exogenous factors such as progestins given to the mother during pregnancy have been postulated to be an etiological factor in some cases of hypospadias (1).

The incidence of other malformations associated with hypospadias is high, 18–32% (11, 36). Cryptorchidism was found to be the most commonly associated malformation, recorded in 3–19% of the patients (6, 9, 11, 28, 31, 34, 36). The incidence of cryptorchidism in boys of one year of age in the normal population has been estimated to about 0.7% (29). Farkas (12), reporting 505 cases of hypospadias, found that cryptorchidism was present in 13%, hypoplasia of the penis in 35% and bifid

Table 2. *Number of recorded associated malformations in the external genitalia in different types of hypospadias*

n = number of patients. *r* = Sperman's rank correlation coefficient

Type of hypospadias	Cryptorchidism		Bifid scrotum		Hypoplasia of penis	
	n	%	*n*	%	*n*	%
Glandular	7	3	1	0.5	8	4
Distal penile	7	4	–	–	5	3
Mid penile	4	4	1	1	6	6
Proximal penile	6	14	2	5	5	12
Penoscrotal	2	5	4	10	9	21
Scrotal	3	27	3	28	2	18
Perineal	7	32	11	50	15	68
	36 (6%)		22 (4%)		50 (8%)	
	(r = 0.96, p<0.02)		(r = 0.85, p<0.05)		(r = 0.93, p<0.02)	

Table 3 *Number of recorded malformations in the lower urinary tract in different types of hypospadias found at voiding cystourethrography*

Type of hypospadias	No of patients examined	Ve ico ureteral reflux	Urethral folds		Urethral recesses
			Obstruc tive	Non obstructive	
Glandular	17	?	–	5	1
Distal penile	?8	3	3	5	–
Mid penile	9	1	1	?	1
Proximal penile	6	–	?	1	1
Penoscrotal	16	–	–	7	3
Scrotal	4	–	–	–	3
Perineal	4	–	1	–	?
	84	6	7	0	11

scrotum in 22% Farkas also found that the incidence of associated anomalies was highest in patients with severe forms of hypospadias

The aim of this retrospective study was to review a series of patients with hypospadias and the presence of associated malformations mainly as a background for studies on the etiology of hypospadias and the late somatic and psychological sequelae of this genital abnormality

CLINICAL MATERIAL

During the period 195?–197? 6 5 boys were admitted to the Department of Paediatric Surgery Karolinska sjukhuset for treatment of hypospadias Excluded from this study are patients with insignificant lesions not considered to require admittance for surgical correction

The different degrees of hypospadias included in the survey are li ted in Table 1 Operations (meatotomy correction of chordee and urethroplasty) were carried out by several surgeons using techniques described by Sir Denis Browne (5) or by Crawford (8) Surgical treatment was not yet completed in all patients at the end of 197_ The median ages for meatotomy correction of chordee and urethropla ty were 5 3 9 and 5 years respectively

Table 4 *Associated malformations recorded different types of hypospadias*

Type of malformation	No of patients with the malforma tion	Type of hypospadias
Ventricular septal defect	1	Glandular
Deafness	1	Distal penile
VOC anal atresia	?	Glandular
Hydrocephalus		Penoscrotal
Omphalocele	1	Glandular
Fsophageal atresia	1	Glandular
An l atresia	1	Mid penile
		Mid penile

ASSOCIATED MALFORMATIONS

All figures in this study are based on records from patients during a 1 year period In this kind of retrospective study there is a certain risk of under-estimation of associated malformations since these may not always have been properly recorded

Cryptorchidism was recorded in 36 patients (6%) bilateral in ?3 cases (4%) The scrotum was bifid in ?? patients (4%) Hypoplasia of the penis as judged subjectively by the surgeon was noted in 50 cases (8%) Like cryptorchidism and bifid scrotum hypoplasia of the penis was found more often in the more severe forms of hypospadias (Table ?)

The lower urinary tract was examined by voiding cystourethrography in 84 patients (1 %) and of these 50% had some form of abnormality (Table 3)

Vesicoureteric reflux was seen in 6 patients Transverse fold formations in the anterior wall of the prostatic urethra just opposite to the verumontanum were observed in 5 patients Of these folds five were slightly obstructive True obstructive posterior urethral valves were observed in two patients

Recesses of various sizes in the posterior wall of the prostatic and bulbous urethra were visualized at voiding cystourethrography in 11 patients It could not be determined whether these recesses represented a partially developed vagina or dilated ducts of the bulbo urethral glands (??) However in one patient with scrotal hypospadias the pouch continued directly into a uterus Biopsies from the gonads of this patient showed both testicular and ovarian tissue The urethral pouches were common in the more severe cases of hypospadias (Table 3)

Table 5 Patients with hypospadias in which chromosome studies were performed

Type of hypospadias	Cryptorchidism	Chromosome constitution
Mid penile	No	46 XY
Mid penile	No	46 XY
Proximal penile	Bilateral	45 XO/46 XY
Penoscrotal	No	46 XY
Penoscrotal	Bilateral	46 XY
Penoscrotal	No	46 XY
Penoscrotal	No	46 XY
Penoscrotal	No	46 XY
Scrotal	No	46 XY
Scrotal	Bilateral	46 XY
Scrotal	No	45 XO/46 XY/47 XYY

Other abnormalities noted at voiding cystourethrography were uracal remnant (one) urethral diverticulum (one) absence of verumontanum (two) neurogenic bladder (one) and duplication of the ureters (one)

Intravenous pyelography was performed in only 10 patients five of whom had slight hydronephrosis not requiring surgery

Various other concomitant malformations are listed in Table 4 Seventeen patients were considered to have an intersex condition In 11 patients chromosome studies were performed two of which had chromosome aberrations 45 XO/46 XY/47 XYY and 45 XO/46 XY as shown in Table 5 The details of the former patient is published elsewhere (13) One patient with scrotal hypospadias had gonads containing both ovarian and testicular structure and a rudimentary uterus and vagina Two patients with distal penile and perineal type of hypospadias had remnants of vagina and uterus removed at operation However the latter three patients were treated before karyotyping became available

DISCUSSION

The differentiation of the fetus into an individual of male status starts early in embryonic life when the indifferent gonad is developed into a testis at a gestational age of 7 weeks (20) The external genitalia start to differentiate at the same time as Leydig cells appear in the testis at the 8th week (20) The initiation of testosterone secretion from the Leydig cells is believed to be stimulated by chorionic gonadotrophin (hCG) from the maternal circulation Later in fetal life about the 12th week the testosterone secretion is controlled by LH from the fetal pituitary gland (21) The plasma and testicular testosterone concentrations rise simultaneously to reach a sharp peak at the 12th week (3 25 27) It is assumed that the development of the external genitalia in the male is dependent upon testosterone production from the testis (20) Testosterone is metabolized in the genital skin to 5α dihydrotestosterone which is *thought to* be the active intracellular androgen in this area (30) The Mullerian structures in the male disappear from the 8th week under the influence of a peptide hormone Mullerian inhibiting hormone (MIH) secreted by the fetal Sertoli cells (17 18)

In the 14 week old fetus the penile corporal part of the urethra is *fully developed* with the meatus at the base of the glans Later in the 4th gestational month formation of the glandular portion of the urethra is completed from an intrusion of an epithelial cord on the ventral side of the glans (16) and the meatus is now in its normal position

In hypospadias the development of the external genitalia is *arrested before* its full differentiation Several of our patients had signs of testicular insufficiency Bifid scrotum and hypoplasia of the penis could be explained by an insufficient secretion of androgens from the testis The presence of remnants of a uterus and a vagina in the males indicates defective testicular activity on the development of the male genital system In cases where remnants of uterus are present in males a defective action of the Mullerian inhibiting hormone is probably the cause Since the regression of the Mullerian structures and the development of the external genitalia are taking place at about the same time in the 60-day old male fetus a connection between the hypospadias and the remnants of the Mullerian structures in the individuals described above is highly suggestive The descent of the testes takes place during the last month of fetal life (29) We found a high incidence of cryptorchidism in this material especially in patients with the more severe forms of hypospadias

One may speculate on a common etiology between some cases of hypospadias and cryptorchidism e g an androgen deficiency affecting both the development of the external genitalia and the descent of the testis although during different stages of fetal development

ACKNOWLEDGEMENT

I am indebted to Professor N O Ericsson for placing his clinical material at my disposal This work was supported in part by the Prenatal Fund and the Swedish Medical Research Council grant 3168

REFERENCES

1 Aarskog D Clinical and cytogenetic studies in hypospadias Acta Paediat Scand Suppl 03 1970
2 Aarskog D Intersex masquerading as simple hypospadias Birth Defects 7 1?? 1971
3 Abramovich D R & Rowe P Foetal plasma testosterone levels at mid pregnancy and at term relationship to foetal sex J Endocrinol 56 6?1 1973
4 Avellan L The incidence of hypospadias in Sweden Scand J Plast Reconstr Surg 9 1?9 1975
5 Browne D Hypospadias Postgrad Med J 25 367 1949
6 Cendron J Treatment of hypospadias Proc R Soc Med 64 1 5 1964
7 Chen Y C & Woolley F V Genetic studies in hypospadias in males J Med Genet 8 153 1971
8 Crawford B S The reconstructive surgery of the urethra with special reference to hypospadias Ann R Coll Surg Engl 41 3? 1967
9 Culp O S Struggles and triumphs with hypospadias and associated anomalies review of 400 cases J Urol 96 339 1966
10 Falconer D S The inheritance of liability to certain diseases estimated from the incidence among relatives Ann Hum Genet 29 51 1965
11 Fallon B Devine C J & Horton C E Congenital anomalies associated with hypospadias J Urol 116 585 1976
12 Farkas L G Hypospadias Czech Publ Acad Sci Prague 1967
13 Fraccaro M Lindsten J Klinger H P Tiepolo L Bergstrand C C Herrlin K M Livaditis A Petrson M & Tillinger K G Cytogenetical and clinical investigations in four subjects with anomalies of sexual development Ann Hum Genet 29 81 1966
14 Griffin J E Punyashthiti K & Wilson J D Comparison of cells from control subjects and from patients with hereditary male pseudohermaphroditism due to androgens resistance J Clin Invest 57 134 1976
15 Hadžiselimović F Chryptorchidism Ultrastructure of normal and cryptorchid testis development Adv Anat Embryol Cell Biol 53 3 1977
16 Jirasek J A Principles of reproductive embryology In J L Simpson (ed) Disorders of sexual differentiation Academic Press Inc New York and London 1976 p 5?
17 Josso N Permeability of membranes to the Mullerian inhibiting substance synthesized by the human fetal testis in vitro A clue to its biochemical nature J Clin Endocrinol Metab 34 65 1972
18 Josso N Muller inhibiting activity of human fetal testicular tissue deprived of germ cells by in vitro irradiation Pediatr Res 8 755 1974
19 Josso N Development and descent of the fetal testis In J R Bierich (ed) Maldescensus testis Urban und Schwarzenberg Munchen Wien and Baltimore 1977 p 3
20 Jost A Vigier B Prépin J & Perchellet J P Studies on sex differentiation in mammals Recent Prog Horm Res 29 1 1973
21 Kaplan S L Grumbach M M & Aubert M L The ontogenesis of pituitary hormones and hypothalamic factors in the human fetus Maturation of central nervous system Regulation of anterior pituitary function Recent Prog Horm Res 32 161 1976
22 Kjellberg S E Ericsson N O & Rudhe U The lower urinary tract in childhood Some correlated clinical and roentgenologic observations Almquist & Wiksell Stockholm 1957 p 33
23 Lowry R B & Kliman M R Hypospadias in successive generations possible dominant gene inheritance Clin Genet 9 85 1976
24 Mainwaring W I P The mechanism of action of androgens Springer Verlag New York Heidelberg and Berlin 1976 p 46
25 Payne A H & Jaffe R B Androgen formation from pregnenolone sulfate by fetal neonatal prepubertal and adult human testes J Clin Endocrinol Metab 40 10? 1975
26 Rajfer J & Walsh P C The incidence of intersexuality in patients with hypospadias and cryptorchidism J Urol 116 769 1976
27 Reyes F J Boroditsky R S Winter J S D & Faiman C Studies on human sexual development II Fetal and maternal serum gonadotropin and sex steroid concentrations J Clin Endocrinol Metab 38 61 1974
28 Ross J F Farmer A W & Lindsay W K Hypospadias A review of 230 cases Plast Reconstr Surg 4 357 1959
29 Scorer C G & Farrington G H Congenital deformities of the testis and epididymis Butterworths London 1971
30 Siiteri P K & Wilson J D Testosterone formation and metabolism during made sexual differentiation in the human embryo J Clin Endocrinol Metab 38 113 1974
31 Sykes P J & Ho L C Y Hypospadias A report on 193 treated cases Plast Reconstr Surg 50 45? 197?
32 Sørensen H R Hypospadias with special reference to etiology E Munksgaard Copenhagen 1953
33 Walsh P C Madden J P Harrod M J Gold

Table 5 *Patients with hypospadias in which chromosome studies were performed*

Type of hypospadias	Cryptorchidism	Chromosome constitution
Mid penile	No	46 XY
Mid penile	No	46 XY
Proximal penile	Bilateral	45 XO/46 XY
Penoscrotal	No	46 XY
Penoscrotal	Bilateral	46 XY
Penoscrotal	No	46 XY
Penoscrotal	No	46 XY
Penoscrotal	No	46 XY
Scrotal	No	46 XY
Scrotal	Bilateral	46 XY
Scrotal	No	45 XO/46 XY/47 XYY

Other abnormalities noted at voiding cystourethrography were urachal remnant (one), urethral diverticulum (one), absence of verumontanum (two), neurogenic bladder (one) and duplication of the ureters (one).

Intravenous pyelography was performed in only 10 patients, five of whom had slight hydronephrosis not requiring surgery.

Various other concomitant malformations are listed in Table 4. Seventeen patients were considered to have an intersex condition. In 11 patients chromosome studies were performed, two of which had chromosome aberrations 45 XO/46 XY/47 XYY and 45 XO/46 XY as shown in Table 5. The details of the former patient is published elsewhere (13). One patient with scrotal hypospadias had gonads containing both ovarian and testicular structure and a rudimentary uterus and vagina. Two patients with distal penile and perineal type of hypospadias had remnants of vagina and uterus removed at operation. However, the latter three patients were treated before karyotyping became available.

DISCUSSION

The differentiation of the fetus into an individual of male status starts early in embryonic life, when the indifferent gonad is developed into a testis at a gestational age of 7 weeks (20). The external genitalia start to differentiate at the same time as Leydig cells appear in the testis at the 8th week (20). The initiation of testosterone secretion from the Leydig cells is believed to be stimulated by chorionic gonadotrophin (hCG) from the maternal circulation. Later in fetal life, about the 12th week, the testosterone secretion is controlled by LH from the fetal pituitary gland (21). The plasma and testicular testosterone concentrations rise simultaneously to reach a sharp peak at the 12th week (3, 25, 27). It is assumed that the development of the external genitalia in the male is dependent upon testosterone production from the testis (20). Testosterone is metabolized in the genital skin to 5α-dihydrotestosterone, which is thought to be the active intracellular androgen in this area (30). The Mullerian structures in the male disappear from the 8th week under the influence of a peptide hormone, Müllerian inhibiting hormone (MIH), secreted by the fetal Sertoli cells (17, 18).

In the 14 week old fetus the penile corporal part of the urethra is fully developed with the meatus at the base of the glans. Later in the 4th gestational month, formation of the glandular portion of the urethra is completed from an intrusion of an epithelial cord on the ventral side of the glans (16), and the meatus is now in its normal position.

In hypospadias the development of the external genitalia is arrested before its full differentiation. Several of our patients had signs of testicular insufficiency. Bifid scrotum and hypoplasia of the penis could be explained by an insufficient secretion of androgens from the testis. The presence of remnants of a uterus and a vagina in *the males indicates* defective testicular activity on the development of the male genital system. In cases where remnants of uterus are present in males a defective action of the Mullerian inhibiting hormone is probably the cause. Since the regression of the Mullerian structures and the development of the external genitalia are taking place at about the same time in the 60 day old male fetus, a connection between the hypospadias and the remnants of the Mullerian structures in the individuals described above is highly suggestive. The descent of the testes takes place during the last month of fetal life (29). We found a high incidence of cryptorchidism in this material, especially in patients with the more severe forms of hypospadias.

Acta Pædiatr Scand 68 593–597 1979

A PROSPECTIVE PSYCHOLOGICAL AND CYTOGENETIC STUDY OF THREE GIRLS WITH MOSAIC MONGOLISM

T LINNE

F om the Departments of Paediatrics and Child Psychiatry Karolinska Institutet St Goran s Child en s Hospital and the Depa tment of Clinical Genetics Karolinska Hospital Stockholm Sweden

ABSTRACT Linne T (Departments of Paediatrics and Child Psychiatry Karolinska Institutet St Goran s Children s Hospital and Department of Clinical Genetics Karolin ka Hospital Stockholm Sweden) A prospective psychological and cytogenetic study of three girls with mosaic mongolism Acta Paediatr Scand 68 593 1979 —Three girls with mosaic mongolism (46 XX/47 XX +21) were followed cytogenetically and psychologically from the time of diagnosis at 8 17 and 32 months of age respectively to the age of 13–15 5 years All showed muscular hypotonia and hyperflexible joints at the time of diagnosis but other wise the physical characteristics of Down s syndrome were weakly expressed The percentage of trisomic cells in the peripheral blood decreased with time but were still higher in lymphocytes than in skin fibroblasts at the last investigation Developmental milestones were delayed in all cases and the developmental and intelligence quotients were decreased Mental retardation was only slight in one of the cases (I Q =65 at the age of 14 years 8 months) The intelligence quotients showed declining trends with time Social ability and school results tended to be better than could be expected from the test results

KEY WORDS Mosaic mongolism mental development chromosome analysis

About 1–2% of all patients with Down s syndrome demonstrate a chromosomal mosaicism (9) Most cases have one cell line with an extra chromosome 21 and one with 46 normal chromosomes Theoretically fewer aberrant cells should mean fewer and less pronounced symptoms of Down s syndrome Early reports also pointed in that direction (2 8) and were supported by the findings of mosaicism among some apparently healthy parents of children with trisomy 21 (10 13) This opinion has later been questioned (7)

In order to get some further information about the mental development and frequency of trisomic cells at different ages in mosaic mongolism three girls have been investigated on several occasions and the results are presented in this report

CASE REPORTS

ie clinical picture of the patients is presented in Fig 1 d Table 1 Data on the mental development are given Table

Case 1 The proposita was the second child of a 6-year old mother and a 31 year-old father Both parents and a brother had had a late psychomotor development The mother walked at 24–26 months the father at 17 months and the brother at 24 months of age The brother s I Q (Terman Merrill) was 65 at the age of 8 years

The girl was born at term in 1958 weight 3 770 g and length 50 cm Abundant skin in the back of the neck was noted at birth Down s syndrome was suspected a few months after birth and she was then admitted to a children s clinic for investigation She had a flat and broad nasal bridge slanting eyes flat occiput abundant skin in the back of the neck generalized muscular hypotonia hyperflexible joints low normal Caffey s index (64) and retarded psychomotor development Chromosome investigation was not done at this time and the definite diagnosis of mosaic mongolism was not made until the age of 6 5 years A faint systolic murmur was heard at that time but ECG and X rays of the heart and lungs were normal EEG showed scattered epileptogenic activity of sharp wave type At the age of 15 5 years EEG showed diffuse abnormalities with slow basic rhythm without epileptogenic activity

The girl's psychomotor development was late She could sit at 11 months and walk at 24–25 months Development and intelligence tests have shown a moderate mental retardation I Q has tended to decrease with increasing age The I Q (Terman Merrill) was 51 and 41 at 6 4 and 15 5 years respectively Since the age of 7 years she has gone to a comprehensive school for mentally retarded

stein J I MacDonald P C & Wilson J D Familiar incomplete male pseudohermaphroditism type 2 Decreased dihydrotestosterone formation in pseudovaginal perineoscrotal hypospadias N Engl J Med 291 944 1974

34 Wehmert J Zur Korrelation zwischen Hypospadie und weiteren angeborenen Missbildungen *Z Urol Nephrol* 67 857 1974

35 Wilson J D & Walther J D The conversion of testosterone to 5α androstan 17β-ol 3-one (dihydrotestosterone) by skin slices of man *J Clin Invest* 48 271 1969

36 Wray R C Ribaudo J M & Weeks P M The Byars hypospadias repair A review of 253 consecutive patients *Plast Reconstr Surg* 58 329 1976

37 Zurbrugg R F Inborn errors in testosterone biosynthesis with special reference to 17-oxo steroid reductase deficiency *Helv Paediatr Acta* Suppl 34 63 1974

Submitted Aug 22 1978
Accepted Dec 21 1978

Department of Paediatric Surgery
Karolinska sjukhuset
S 104 01 Stockholm
Sweden

Acta Pædiatr Scand 68 593–597 1979

A PROSPECTIVE PSYCHOLOGICAL AND CYTOGENETIC STUDY OF THREE GIRLS WITH MOSAIC MONGOLISM

T LINNE

From the Departments of Paed atrics and Child Psychiatry Karolinska Institutet St Goran s Children s Hospital and the Department of Clinical Genetics Karolinska H spital Stockholm Sweden

ABSTRACT Linne T (Departments of Paediatrics and Child Psychiatry Karolinska Institutet St Goran s Children s Hospital and Department of Clinical Cenetics Karolinska Hospital Stockholm Sweden) A prospective psychological and cytogenetic study of three girls with mosaic mongolism Acta Paediatr Scand 68 593 1979.—Three girls with mosaic mongolism (46 XX/47 XX +21) were followed cytogenetically and psychologically from the time of diagnosis at 8 17 and 32 months of age respectively to the age of 13–15 5 years All showed muscular hypotonia and hyperflexible joints at the time of diagnosis but other wise the physical characteristics of Down s syndrome were weakly expressed The percentage of trisomic cells in the peripheral blood decreased with time but were still higher in lymphocytes than in skin fibroblasts at the last investigation Developmental milestones were delayed in all cases and the developmental and intelligence quotients were decreased Mental retardation was only slight in one of the cases (I Q =65 at the age of 14 years 8 months) The intelligence quotients showed declining trends with time Social ability and school results tended to be better than could be expected from the test results

KEY WORDS Mosaic mongolism mental development chromosome analysis

About 1–2% of all patients with Down s syndrome demonstrate a chromosomal mosaicism (9) Most cases have one cell line with an extra chromosome 21 and one with 46 normal chromosomes Theoretically fewer aberrant cells should mean fewer and less pronounced symptoms of Down s syndrome Early reports also pointed in that direction (2 8) and were supported by the findings of mosaicism among some apparently healthy parents of children with trisomy 21 (10 13) This opinion has later been questioned (7)

In order to get some further information about the mental development and frequency of trisomic cells at different ages in mosaic mongolism three girls have been investigated on several occasions and the results are presented in this report

CASE REPORTS

The clinical picture of the patients is presented in Fig 1 and Table 1 Data on the mental development are given in Table

Case 1 The proposita was the second child of a 26-year old mother and a 31 year-old father Both parents and a brother had had a late psychomotor development The mother walked at 24–26 months the father at 17 months and the brother at 24 months of age The brother s I Q (Terman Merrill) was 65 at the age of 8 years

The girl was born at term in 1958 weight 3770 g and length 50 cm Abundant skin in the back of the neck was noted at birth Down s syndrome was suspected a few months after birth and she was then admitted to a children s clinic for investigation She had a flat and broad nasal bridge slanting eyes flat occiput abundant skin in the back of the neck generalized muscular hypotonia hyperflexible joints low normal Caffey s index (64) and retarded psychomotor development Chromosome investigation was not done at this time and the definite diagnosis of mosaic mongolism was not made until the age of 6 5 years A faint systolic murmur was heard at that time but ECG and X rays of the heart and lungs were normal EEG showed scattered epileptogenic activity of sharp wave type At the age of 15 5 years EEG showed diffuse abnormalities with slow basic rhythm without epileptogenic activity

The girl s psychomotor development was late She could sit at 11 months and walk at 24–25 months Development and intelligence tests have shown a moderate mental retardation I Q has tended to decrease with increasing age The I Q (Terman Merrill) was 51 and 41 at 6 4 and 15 5 years respectively Since the age of 7 years she has gone to a comprehensive school for mentally retarded

stein J E MacDonald P C & Wilson J D Familiar incomplete male pseudohermaphroditism type 2 Decreased dihydrotestosterone formation in pseudovaginal perineoscrotal hypospadias *N Engl J Med* 291 944 1974

34 Wehmert J Zur Korrelation zwischen Hypospadie und weiteren angeborenen Missbildungen *Z Urol Nephrol* 67 857 1974

35 Wilson J D & Walther J D The conversion of testosterone to 5α androstan 17β ol 3-one (dihydro testosterone) by skin slices of man *J Clin Invest* 48 271 1969

36 Wray R C Ribando J M & Weeks P M The Byars hypospadias repair A review of 253 consecutive patients *Plast Reconstr Surg* 58 329 1976

37 Zurbrugg R P Inborn errors in testosterone biosynthesis with special reference to 17-oxo steroid reductase deficiency *Helv Paediatr Acta* Suppl 34 63 1974

Submitted Aug 2? 1978
Accepted Dec 21 1978

Department of Paediatric Surgery
Karolinska sjukhuset
S 10401 Stockholm
Sweden

Table 2 *Mental de\elopment*

y=year(s) m=month(s) w=week(s)

	Age	Test	Result of test	
Case 1	31 w	Gesell	Motoric	1 -16 w
			Adaptive behaviour	16-?0 w
			Language	24 w
	6 y 5 m	Terman Merrill	I Q =51	
	7 y 11 m	Terman Merrill	I Q =43	
	11 y 6 m	Terman Merrill	I Q =47	
	15 y 6 m	Terman Merrill	I Q =41	
Case	17 m	Gesell	Rough and fine motoric	9-10 m
			Adaptive behaviour	15-16 m
			Language	15 m
	7 m	Gesell	Rough motoric	-4 m
			Fine motoric	8 m
			Adaptive behaviour	4-?6 m
			Language	?1 m
	14 y 8 m	Terman Merrill	I Q =65	
Case 3	3 m	G sell		21- 4 m
	44 m	Gesell	Rough motoric	21 m
			Fine motoric	4-30 m
			Adaptive behaviour	24-30 m
			Language	?4-30 m
	4 y 9 m	Terman Merrill	I Q =51	
	1? y 11 m	Terman Merrill	I Q =38	

the vitality of the child was good at birth During the first days of life she was cyanotic peripherally the mu cular tonus was decreased and the joints hyperflexible Down s syndrome was not suspected

She was referred to a children s clinic at the age of ? 5 years because of retarded psychomotor development and the diagnosis of mosaic mongolism was made Physical findings of mongolism were slight slanting of the palpebral fissures often protruding tongue generalized muscular hypotonia and hyperflexible joints ECG showed 1st

Table 3 *Chromosomal anal\ses of cells from peripheral blood (B) and skin (S)*

	Age	Tissue	Chromosome count 44	45	46	47		Total
Case 1	6 5 y	B	-	?	4	10 (19%)	33 (16 5%)	54
		B	-	?	51	13 (0%		66
		B	-	-	70	10 (13%)		80
		S	-	3	46	4 (7%)		53
	15 5 y	B	-	-	46	4 (8%)		50
		S	-	-	85	2 (1 1%)		87
Case	1 5 y	B	-	-	43	54 (56%)		97
		S	?	3	13	13 (4?%)	6 (?7%)	31
		S	-	-	?1	3 (13%)		24
		S	-	5	1?0	46 (7%)		171
	14 7 y	B	-	-	33	17 (34%)		50
		S	-	-	39	11 (? %)		50
Case 3	y	B	-	-	71	54 (43%)		125
	13 y	B	-	-	9	7 (19%)		36
	13 y	S	-	-	44	6 (1?%)		50

47 XX +D

47 XX +G

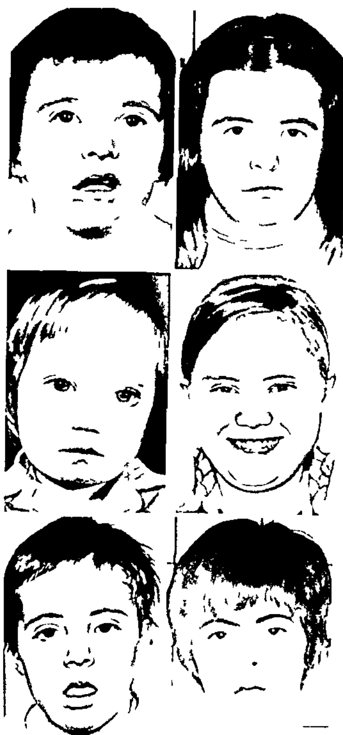


Fig 1 Case 1 2 and 3 at the ages of 6 y 6 m and 15 y 6 m 2 y 3 m and 14 y 6 m and 3 y 11 m and 13 y respectively

Table 1 *Physical stigmata of Downs syndrome*

++ Clearly present + slightly expressed − absent ● no information

	Case		
	1	2	3
Age at examination	6 y 6 m	2 y 3 m	3 y 11 m
Flattened occiput	++	●	●
Abundant neck skin	++	−	−
Oblique palpebral fissures	−	++	+
Epicanthal folds	−	+	−
Dysplastic ears	++	−	−
Flattened nasal bridge	+	++	−
Habitually open mouth	++	++	++
Large tongue	−	−	−
Protruding tongue	+	+	++
Irregular teeth	+	●	−
Short broad hands	+	−	−
Short curved 5th finger	−	−	−
Four finger crease	−	−	−
Increased spacing between 1st and 2nd toe	−	++	−
Muscular hypotonia	++	++	++
Hyperflexible joints	++	++	++

children but transfer to a training school has been considered

Case 2 Previously reported up to the age of 27 months (8) The proposita was the first child of a 38 5 year old mother and a 29 year old father She was born after 40 weeks gestation birthweight 2210 g and length 46 cm She was observed at a children s hospital for a few days after birth because of moderate jaundice and because she was lazy and did not suck satisfactorily A slight generalized muscular hypotonia was noted but no obvious characteristics of mongolism

At the age of 17 months she was investigated because of frequent upper respiratory tract infections and a suspicion of Down s syndrome Physical examination showed a flat nasal bridge slanting palpebral fissures epicanthal fold on one side often protruding tongue generalized muscular hypotonia and hyperflexible joints Caffey s index was low normal (65) There were no signs of heart malformation Chromosome analysis showed a mosaicism with normal and trisomy 21 cells Clinical investigations at 27 months and 5 5 years respectively showed the same principal physical findings as at 17 months EEG at 14 5 years was essentially normal

The psychomotor development was late She supported her head at 5–6 months and walked at 23 months Developmental tests showed slight mental retardation and at the age of 27 months the test results even pointed to a nearly normal intelligence As in the other cases the result showed a declining trend with age I Q (Terman Merrill) was 65 at 14 7 years of age After one year in pre school she started comprehensive school in a class for slow learners i e at the age of 8 years She has done well in school and has obtained average marks

Case 3 The proposita was the first of two children to a 23 5 year old mother and a 31 year old father The length of gestation was 40 weeks birthweight 3 110 g and length 52 cm The amniotic fluid contained meconium but

Table 2 *Mental development*

y=year(s) m=month(s) w=week(s)

	Age	Test	Result of test	
Case 1	31 w	Gesell	Motoric	12–16 w
			Adaptive behaviour	16–20 w
			Language	24 w
	6 y 5 m	Terman Merrill	I Q =51	
	7 y 11 m	Terman Merrill	I Q =43	
	11 y 6 m	Terman Merrill	I Q =47	
	15 y 6 m	Terman Merrill	I Q =41	
Case 2	17 m	Gesell	Rough and fine motoric	9–10 m
			Adaptive behaviour	15–16 m
			Language	15 m
	27 m	Gesell	Rough motoric	24 m
			Fine motoric	8 m
			Adaptive behaviour	24– 6 m
			Language	21 m
	14 y 8 m	Terman Merrill	I Q =65	
Case 3	3 m	Gesell		21–24 m
	44 m	Gesell	Rough motoric	21 m
			Fine motoric	4–30 m
			Adaptive behaviour	4–30 m
			Language	24–30 m
	4 y 9 m	Terman Merrill	I Q =51	
	12 y 11 m	Terman Merrill	I Q =38	

the vitality of the child was good at birth During the first days of life she was cyanotic peripherally the muscular tonus was decreased and the joints hyperflexible Down s syndrome was not suspected

She was referred to a children s clinic at the age of 2 5 years because of retarded psychomotor development and the diagnosis of mosaic mongolism was made Physical findings of mongolism were slight slanting of the palpebral fissures often protruding tongue generalized muscular hypotonia and hyperflexible joints ECG showed 1st

Table 3 *Chromosomal analyses of cells from peripheral blood (B) and skin (S)*

	Age	Tissue	Chromosome count 44	45	46	47		Total
Case 1	6 5 y	B	–		4	10 (19%)	33 (16 5%)	54
		B	–	2	51	13 (0%		66
		B	–	–	70	10 (13%)		80
		S	–	3	46	4 (7%)		53
	15 5 y	B	–	–	46	4 (8%)		50
		S	–	–	85	[illegible] (1 1%)		87
Case	1 5 y	B	–	–	43	54 (56%)		97
		S	2	3	13	13 (42%)	6 (27%)	31
		S	–	–	21	3 (13%)		24
		S	–	5	1 0	46 (27%)		171
	14 7 y	B	–	–	33	17 (34%)		50
		S	–	–	39	11 (22%)		50
Case 3	y	B	–	–	71	54 (43%)		125
	13 y	B	–	–	9	7 (19%)		36
	13 y	S	–	–	44	6 (12%)		50

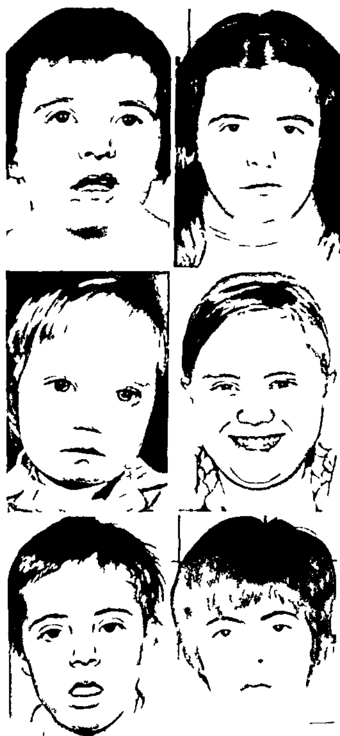


Fig 1 Case 1 2 and 3 at the ages of 6 y 6 m and 15 y 6 m 2 y 3 m and 14 y 6 m and 3 y 11 m and 13 y respectively

children but transfer to a training school has been considered

Case 2 Previously reported up to the age of 27 months (8) The proposita was the first child of a 38 5 year old mother and a 29 year old father She was born after 40 weeks gestation birthweight 2210 g and length 46 cm She was observed at a children s hospital for a few days after birth because of moderate jaundice and because she

Table 1 *Physical stigmata of Down s syndrome*

++ Clearly present + slightly expressed – absent
● no information

	Case		
	1	2	3
Age at examination	6 y 6 m	2 y 3 m	3 y 11 m
Flattened occiput	++	●	●
Abundant neck skin	++	–	–
Oblique palpebral fissures	–	++	+
Epicanthal folds	–	+	–
Dysplastic ears	++	–	–
Flattened nasal bridge	+	++	–
Habitually open mouth	++	++	++
Large tongue	–	–	–
Protruding tongue	+	+	++
Irregular teeth	+	●	–
Short broad hands	+	–	–
Short curved 5th finger	–	–	–
Four finger crease	–	–	–
Increased spacing between 1st and 2nd toe	–	++	–
Muscular hypotonia	++	++	++
Hyperflexible joints	++	++	++

was lazy and did not suck satisfactorily A slight generalized muscular hypotonia was noted but no obvious characteristics of mongolism

At the age of 17 months she was investigated becau of frequent upper respiratory tract infections and a suspicion of Down s syndrome Physical examination showed flat nasal bridge slanting palpebral fissures epicanthal fold on one side often protruding tongue generalized muscular hypotonia and hyperflexible joints Caffey index was low normal (65) There were no signs of heart malformation Chromosome analysis showed a mosaicism with normal and trisomy 21 cells Clinical investigation at 27 months and 5 5 years respectively showed the same principal physical findings as at 17 months EEG at 14 years was essentially normal

The psychomotor development was late She supported her head at 5–6 months and walked at 23 months Developmental tests showed slight mental retardation and at the age of 27 months the test results even pointed to nearly normal intelligence As in the other cases the results showed a declining trend with age I Q (Terman Merrill) was 65 at 14 7 years of age After one year in pre school she started comprehensive school in a class for slow learners i e at the age of 8 years She has done well in school and has obtained average marks

Case 3 The proposita was the first of two children to a 23 5 year old mother and a 31 year old father The length of gestation was 40 weeks birthweight 3 110 g and length 52 cm The amniotic fluid contained meconium but

ACKNOWLEDGMENT

This study was supported by grants from the Swedish Medical Research Council (3681)

REFERENCES

1 Carr J Mental and motor development in young mongol children J Ment Defic Res 14 205 1970
2 Clarke C M Edwards J H & Smallpeice V 21 trisomy/normal mosaicism in an intelligent child with some mongoloid characters Lancet I 10 8 1961
3 Clarke C M Ford C E Edwards J H & Smallpeice V 21 trisomy/normal mosaicism in an intelligent child with some mongoloid characters Lancet II 1? 9 1963
4 Fishler K Koch R & Donnell G N Comparison of mental development in individuals with mosaic and trisomy 21 Down s syndrome Pediatrics 58 744 1976
5 Johnson R C & Abelson R B Intellectual behavioral and physical characteristics associated with trisomy translocation and mosaic types of Down s syndrome Am J Ment Defic 73 852 1969
6 Koch R Share J Webb A & Graliker B V The predictability of Gesell developmental scales in mongolism J Pediatr 6 93 1963
7 Kohn G Taysi K Atkins T E & Mellman W J Mosaic mongolism I Clinical correlations J Pediatr 76 874 1970
8 Lindsten J Alvin A Gustavson K H & Fraccaro M Chromosomal mosaic in a girl with some features of mongolism Cytogenetics 1 0 196?
9 Richards B W Mosaic mongolism J Ment Defic Res 13 66 1969
10 Smith D W Therman E M Patau K A & Inhorn S L Mosaicism in mother of two mongoloids Am J Dis Child 104 534 196?
11 Taylor A I Cell selection in vivo in normal/G trisomic mosaics Nature 219 10 8 1968
12 Taysi K Kohn G & Mellman W J Mosaic mongolism II Cytogenetic studies J Pediatr 76 880 1970
13 Weinstein E D & Warkany J Maternal mosaicism and Down s syndrome (mongolism) J Pediatr 63 599 1963
14 Zeaman D & House B J Mongoloid M A is proportional to log C A Child Develop 33 481 1962

Submitted Oct 30 1978
Accepted Nov 28 1978

Department of Paediatrics Karolinska Institutet
St Goran s Children s Hospital
S 11281 Stockholm
Sweden

degree of AV block and EEG marked delta activity occipitally At the age of 13 years EEG was apparently normal

The psychomotor development was late She sat at 10 months and walked at 24 months Development and intelligence tests have shown a moderate mental retardation but with age the quotients showed a declining trend At the ages of 2 7 and 3 7 years the development quotient (Gesell) was 70 and 58 respectively and at 4 7 and 12 9 years the I Q (Terman Merrill) 51 and 38 respectively After one and a half years in pre school she started comprehensive school for mentally retarded children at the age of 8 years She was transferred to a training school after two years because of difficulties in learning The social adjustment has been very good

CYTOGENETIC STUDIES

The results of the chromosome analysis were essentially the same in all cases Two cell lines with 46 and 47 chromosomes respectively were found both in cultured lymphocytes and skin fibroblasts (Table 3) The cells with 46 chromosomes had a normal female karyotype while those with 47 chromosomes were trisomic for chromosome 21 (Q band analysis) The percentage of trisomy 21 cells was higher in the lymphocytes than in the skin fibroblasts at all investigations and decreased with increasing age of the patients both in the lymphocytes and the skin fibroblasts

Chromosome analysis of the parents and sibs showed normal karyotypes

DISCUSSION

Only a few reports of longitudinally followed cases with mosaic mongolism have been published (2 3 4 7 11 12) None of these cases was followed for as long a time as those in the present study The majority of the previously reported cases as well as the present three girls have shown a decrease in the frequency of trisomic cells in the peripheral blood and in skin fibroblasts The frequency of trisomic cells was consistently higher in blood than in skin in all three patients In contrast all cases of Taysi et al (12) except one had a higher frequency of trisomic cells in skin fibroblasts Selection for trisomic cells in long term skin cultures occurs (12) but should only modify the figures Probably the frequency of trisomic cells differs from tissue to tissue and the decreasing frequency reflects selection against the trisomic cell line

The mental retardation in the present three girls progressed Thus with increasing chronological age the development and intelligence quotient showed a declining trend This is in accordance with what has been reported in non mosaic (4 6 14) as well as in mosaic mongolism (3 4 7) Moreover the motorics in early age as in non mosaic mongolism (1) tended to be more retarded than the mental development Thus the mosaicism does not seem to change the general pattern of mental development as seen in non mosaic mongolism

There was a poor correlation between physical stigmata of Down s syndrome (5) frequency of trisomic cells (4 12) and the degree of mental retardation in accordance with previous reports of mosaic mongolism Case 1 had a low frequency of trisomic cells in the blood as well as in the skin markedly lower than in the other cases Despite of that the physical features of Down s syndrome were at least as much expressed as in the other patients and the I Q was low and declined with increasing chronological age However additional factors might have played a role in this case since the girl s parents and brother also showed a late psychomotor development In contrast case 2 had the highest frequency of trisomic cells in blood as well as in skin but she was only slightly mentally retarded

Although the individuals with mosaic mongolism as a group have a better prognosis of mental development (4 7) the overlapping in the distributions of the I Q in non mosaic and mosaic mongolism is very high and as seen from cases 1 and 3 the prognosis is not necessarily better in mosaic than in non mosaic mongolism More exceptional cases such as case 2 who had an I Q of 65 at the age of 14 7 years are of course important to remember when talking to parents of a baby with mosaic

Acta Pædiatr Scand 68 599–604 1979

ADRENOCORTICAL FUNCTION IN PUBERTY

Serum ACTH Cortisol and Dehydroepiandrosterone in Girls and Boys

D APTER A PAKARINEN G L HAMMOND and R VIHKO

From the Department of Medical Chemistry University of Helsinki Helsinki and The Department of Clinical Chemistry University of Oulu Oulu Finland

ABSTRACT Apter D Pakarinen A Hammond G L and Vihko R (Department of Medical Chemistry University of Helsinki Helsinki and Department of Clinical Chemistry University of Oulu Oulu Finland) Adrenocortical Function in Puberty Serum ACTH cortisol and dehydroepiandrosterone in girls and boys Acta Paediatr Scand 68 599 1979 —Serum ACTH cortisol and dehydroepiandrosterone (DHEA) were determined in 200 girls and 80 boys In girls serum DHEA showed significant increases between all bone age groups from the youngest one 7 5 years to 12 5 years A plateau was then seen up to 15 5 years of age followed by a continuous increase to the oldest group (18 5 years) In boys a progressive increase in DHEA was also seen from the youngest age group (8 5 years) but a period of a more rapid increase did not commence until after 12 5 years of age and it then continued to the oldest group The level of DHEA in boys was significantly lower than in girls until the oldest group in concert with the earlier pubertal development in girls The importance of DHEA in initiating the early physical signs of normal puberty seems also to be different in the two sexes since serum DHEA in girls was almost double that seen in boys when compared according to the stage of pubic hair growth Serum cortisol showed a small progressive increase in girls the concentrations postmenarche being significantly higher than premenarche In boys a decrease was seen up to 12 5 years of age and an increase occurred from 16.5 years onwards In both sexes ACTH and cortisol levels showed an inverse but non-significant relationship to each other Serum ACTH levels in the different age groups showed no significant changes

KEY WORDS Child development menarche radioimmunoassay ACTH cortisol dehydroepiandrosterone

The consensus of opinion seems to be that one of the earliest hormonal changes preceding the physical changes of puberty is activation of the adrenal cortex This has been concluded from the increasing serum dehydroepiandrosterone and dehydroepiandrosterone sulphate concentrations observed as early as about 6 years of age in boys and girls (7 8 14–17) However during puberty very little is known about the secretion of cortisol the main corticoid secreted by the adrenal The purpose of this study was to investigate adrenal function and its regulation in relation to pubertal changes We report the concentrations of serum ACTH cortisol and dehydroepiandrosterone in 200 [illegible] 0 boys The boys were examined on three occasions at one year intervals and the girls twice at 1 5 year intervals

MATERIALS AND METHODS

Subjects Details of the female (3 4) and male (3) population have already been described as well as the measurement of the physical parameters used (9 18) In postmenarcheal girls samples were obtained at days 6–9 of the cycle All the samples were drawn at 08 00–10 00 a m In girls bone age determination was carried out at each examination and therefore the data on girls are given in relation to bone age In boys bone age was not regularly recorded and the data are therefore given in relation to chronological age

Hormone determinations The estimation of serum ACTH was performed using CEA IRE SORIN (CIS) kits (Departement des Rayonnements Ionisants B P No 21 91190 Gif Sur Yvette France) The methodo

Acta Pædiatr Scand 68 599–604 1979

ADRENOCORTICAL FUNCTION IN PUBERTY

Serum ACTH Cortisol and Dehydroepiandrosterone in Girls and Boys

D APTER A PAKARINEN G L HAMMOND and R VIHKO

From the Department of Medical Chemistry University of Helsinki Helsinki and The Department of Clinical Chemistry University of Oulu Oulu Finland

ABSTRACT Apter D Pakarinen A Hammond G L and Vihko R (Department of Medical Chemistry University of Helsinki Helsinki and Department of Clinical Chemistry University of Oulu Oulu Finland) Adrenocortical Function in Puberty Serum ACTH cortisol and dehydroepiandrosterone in girls and boys Acta Paediatr Scand 68 599 1979.—Serum ACTH cortisol and dehydroepiandrosterone (DHEA) were determined in 200 girls and 80 boys In girls serum DHEA showed significant increases between all bone age groups from the youngest one 7 5 years to 12 5 years A plateau was then seen up to 15 5 years of age followed by a continuous increase to the oldest group (18 5 years) In boys a progressive increase in DHEA was also seen from the youngest age group (8 5 years) but a period of a more rapid increase did not commence until after 12 5 years of age and it then continued to the oldest group The level of DHEA in boys was significantly lower than in girls until the oldest group in concert with the earlier pubertal development in girls The importance of DHEA in initiating the early physical signs of normal puberty seems also to be different in the two sexes since serum DHEA in girls was almost double that seen in boys when compared according to the stage of pubic hair growth Serum cortisol showed a small progressive increase in girls the concentrations postmenarche being significantly higher than premenarche In boys a decrease was seen up to 12 5 years of age and an increase occurred from 16.5 years onwards In both sexes ACTH and cortisol levels showed an inverse but non-significant relationship to each other Serum ACTH levels in the different age groups showed no significant changes

KEY WORDS Child development menarche radioimmunoassay ACTH cortisol dehydroepiandrosterone

The consensus of opinion seems to be that one of the earliest hormonal changes preceding the physical changes of puberty is activation of the adrenal cortex This has been concluded from the increasing serum dehydroepiandrosterone and dehydroepiandrosterone sulphate concentrations observed as early as about 6 years of age in boys and girls (7 8 14–17) However during puberty very little is known about the secretion of cortisol the main corticoid secreted by the adrenal The purpose of this study was to investigate adrenal function and its regulation in relation to pubertal changes We report the concentrations of serum ACTH cortisol and dehydroepiandrosterone in 200 girls and 80 boys The boys were examined on three occasions at one year intervals and the girls twice at 1 5 year intervals

MATERIALS AND METHODS

Subjects Details of the female (3 4) and male (3) population have already been described as well as the measurement of the physical parameters used (9 18) In postmenarcheal girls samples were obtained at days 6–9 of the cycle All the samples were drawn at 08 00–10 00 a m In girls bone age determination was carried out at each examination and therefore the data on girls are given in relation to bone age In boys bone age was not regularly recorded and the data are therefore given in relation to chronological age

Hormone determinations The estimation of serum ACTH was performed using CEA IRE SORIN (CIS) kits (Departement des Rayonnements Ionisants B P No 21 91190 Gif Sur Yvette France) The methodo-

Table 2 *Serum DHEA cortisol and ACTH in bo\s according to chronological age*

	Age (years)										
	8 5	9 5	10 5	11 5	1 5	13 5	14 5	15 5	16 5	17 5	18 0–19 9
No of subje ts	7	14	18	_?	18	3	16	19	18	17	14
DHEA µg/l											
Mean	0 55	1 09	1 0	1 70	1 66	2 44	37	3 14	3 56	4 69	6 41
S E M	0 1?	0 ?4	0 17	0 3?	0 24	0 36	0 25	0 47	0 57	0 60	0 48
Cortisol µg/l											
Mean	87	99	10	86	64	64	60	66	6?	101	1 0
S E M	11	1?	9	10	11	8	10	10	11	10	8
ACTH ng/l											
Mean	37	65	105	140	140	10_	48	55	67	64	54
S E M	11	?0	2?	40	48	31	8	13	2?	19	8
Median	35	38	5?	50	48	49	47	48	48	44	46

$p<0.05$ $p<0.01$ $p<0.001$

culated for each individual The mean annual changes are calculated and then consecutively added to or subtracted from the value of the previous age group beginning from the actual mean concentration of the age group of 13 5 years which included the largest number of subjects Table 1 gives the actual mean con centrations of all the samples analyzed in annual bone age groups for the three hormones in a cross sectional manner

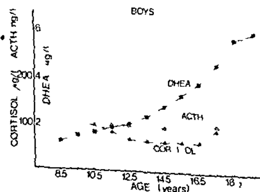


Fig Serum DHEA cortisol and ACTH in pubertal boys The changes have been expressed in a mixed longi tudinal way (see ref 10) In this case the means of the individual annual changes are successively added to or subtracted from the mean actual con entration of the age group of 13 5 years This point i the one representing the actual mean of the age group others reflect relative changes

DHEA shows an increase in the youngest age groups of girls (Fig 1 Table 1) Its con centration is significantly increased up to 12 5 years of age after which a plateau is seen which extends until 15 5 years of age The plateau is again followed by a continuous in

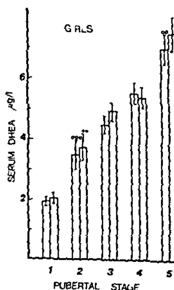


Fig 3 Serum DHEA according to breast (light columns) and pubic hair (dark columns) stages in girls One to three of the symbols O or × indicate $p<0.05$ $p<0.01$ $p<0.001$ significances of difference in respect to the preceding stage of breast and pubic hair de elopment respectively The bars indicate ±1 standard error of the mean The number of subjects in each group is ?9–54 girls

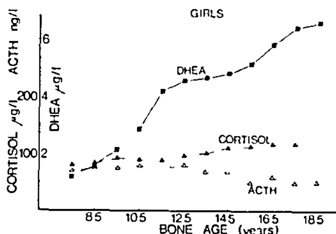


Fig. 1. Serum DHEA, cortisol and ACTH in pubertal girls. The changes have been expressed in a mixed longitudinal manner (see ref. 10), in which the ratios of changes in hormone concentrations and bone age in each individual are first calculated. The mean annual changes are then added to or subtracted from the value of the previous age group beginning from the mean actual concentration of the age group of 13.5 years, which included the largest number of subjects. This point is the one representing the actual mean of the age group, others reflect relative changes.

logies outlined in the manufacturer's instructions were followed and 0.1–0.2 ml samples of serum were used. Individual assays were monitored by the inclusion of low, medium and high controls in each batch of unknown samples. Each batch also included samples from all age groups, and all samples from the same person were determined in the same series. In our hands, the lowest detectable amount which could be measured reproducibly was 7.2 ng/l when 0.1 ml of serum was used. The intra assay coefficients of variation were 15% (21 ng/l), 11% (72 ng/l) and 7% (310 ng/l). The interassay coefficient of variation was 17% (68 ng/l, $n=$[illegible]).

Serum cortisol was determined by a highly specific method as previously described (2). In short, the method is as follows: Serum (0.1 ml) is diluted with water, extracted with diethyl ether/ethyl acetate (90:10 by vol.) and chromatographed on a microcolumn of Lipidex 5000™ (hydroxyalkoxypropyl Sephadex). Part of the cortisol fraction was taken for radioimmunoassay using a highly specific antibody prepared against cortisol-21-hemisuccinate-bovine serum albumin (BSA) as the antigen.

Dehydroepiandrosterone was determined in duplicate 50 µl serum samples after extraction with 1 ml of petroleum ether and using an automated approach similar to that for some sex steroids as outlined previously (11).

The antibody was raised against 3β-hydroxy-7-(0-carboxymethyl)oxime-5-androsten-17-one-BSA. Characteristics of the antibody and details of the radioimmunoassay of dehydroepiandrosterone are described in Hammond et al. (12).

The statistical treatment of the results was performed at the Department of Data Processing, University of Oulu. Groups were compared using the two-tailed unpaired Student's *t* test or the Wilcoxon test for pair differences.

RESULTS

Fig. 1 shows the changes in serum ACTH, cortisol and dehydroepiandrosterone (DHEA) in girls, expressed by a mixed longitudinal approach (modified from Gupta 10). In this approach, the ratios of individual changes in hormone concentration and bone age are cal-

Table 1. *Serum DHEA, cortisol and ACTH in girls according to bone age*

In postmenarcheal girls, the values are from samples taken between days 6–9 of the menstrual cycle

	Bone age (years)											
	7.5	8.5	9.5	10.5	11.5	12.5	13.5	14.5	15.5	16.5	17.5	18.5
No. of subjects	7	16	12	27	18	24	33	18	28	33	25	7
DHEA µg/l (significance brackets: 7.5–8.5 *; 8.5–10.5 *; 10.5–12.5 **; 13.5–16.5)												
Mean	0.87	1.34	1.55	2.68	3.49	4.60	4.78	5.10	4.9[illegible]	6.94	7.30	6.93
S.E.M.	0.11	0.14	0.12	0.20	0.56	0.48	0.79	0.51	0.5[illegible]	0.66	0.83	0.58
Cortisol µg/l (significance brackets: 9.5–14.5 *; 13.5–16.5)												
Mean	82	80	83	99	87	100	103	121	116	132	130	128
S.E.M.	15	7	8	8	10	7	7	10	7	6	8	14
ACTH ng/l												
Mean	85	68	59	64	118	82	69	88	81	63	60	50
S.E.M.	16	11	9	6	33	13	10	14	10	6	5	14
Median	69	64	52	64	59	63	55	67	62	50	54	36

$^{*}p<0.05$ $^{**}p<0.01$ $^{***}p<0.001$

of normal puberty seems also to be different in the two sexes In the case of pubic hair (Figs 3 and 4) the level of serum DHEA in girls is almost double (160–180%) that seen in boys at each stage Quantitatively greater increases in testosterone or a higher sensitivity of the target tissues for androgenic stimulus in boys may be responsible for this sex difference

The changes in serum cortisol are not very marked In girls a progressive but slow increase is observed throughout the age groups studied The changes seen in boys although small follow a somewhat different pattern Serum cortisol first displays a significant decrease until about 12 years of age and an increase takes place after 16 5 years of age The serum cortisol concentrations found by us are close to those recently reported by Ducharme et al (8) Their results also show similar patterns of pubertal changes in serum cortisol Quite recently Parker et al have presented data showing a tendency to increasing serum cortisol concentrations in the course of puberty but the changes were not statistically significant (15) Kenny et al (13) found an increase in cortisol production rate in the course of puberty but only in proportion to the increase in body size

Only small and statistically insignificant changes are seen in serum ACTH The changes seem to be inversely related to those of serum cortisol

On the basis of the data obtained early activation of the adrenal cortex preceding puberty involves only a restricted part of its activities and so far has been reported to be reflected only in the secretion of DHEA (and its sulphate) (7 8 14–17) and pregnenolone (4) It is important to emphasize that there is a sex difference in this function as found in this and an earlier study (8) Serum FSH and prolactin also display changes which are different in the two sexes and herald the onset of puberty (3) The interaction of prolactin and the adrenal cortex in puberty is possible because elevated prolactin production leads to elevated serum DHEA which returns to normal along with the normalization of serum prolactin (5 6 19) However the small physiological changes of serum prolactin in infants and during the years prior to puberty especially in boys (1) make it unlikely that prolactin alone is of importance in the initiation of puberty

REFERENCES

1 Aubert M L Sizonenko P C Kaplan S L & Grumbach M M The ontogenesis of human prolactin from fetal life to puberty *In* P G Crosignani & C Robyn (eds) *Prolactin and human reproduction* Academic Press London New York San Francisco 1977 p 9

2 Apter D Janne O & Vihko R Lipidex chromatography in the radioimmunoassay of serum and urinary cortisol *Clin Chim Acta 63* 139 1975

3 Apter D Pakarinen A & Vihko R Serum prolactin FSH and LH during puberty in girls and boys *Acta Pediatr Scand 67* 417 1978

4 Apter D & Vihko R Serum pregnenolone progesterone 17 hydroxyprogesterone testosterone and 5α-dihydrotestosterone during female puberty *J Clin Endocrinol Metab 45* 1039 1977

5 Bassi F Giusti G Borsi L Cattaneo S Giannotti P Forti G Pazzagli M Vigiani C & Serio M Plasma androgens in women with hyperprolactinemic amenorrhoea *Clin Endocrinol 6* 5 1977

6 Carter J N Tyson J E Warne G L McNeilly A S Faiman C & Friesen H G Adrenocortical function in hyperprolactinemic women *J Clin Endocrinol Metab 45* 973 1977

7 de Peretti E & Forest M G Unconjugated dehydroepiandrosterone plasma levels in normal subjects from birth to adolescence in human The use of a sensitive radioimmunoassay *J Clin Endocrinol Metab 43* 98 1976

8 Ducharme J R Forest M G de Peretti E Sempe M Collu R & Bertrand J Plasma adrenal and gonadal sex steroids in human pubertal development *J Clin Endocrinol Metab 42* 468 1976

9 Greulich W W & Pyle S J *Radiographic atlas of skeletal development of the hand and wrist* Stanford University Press Stanford 1959

10 Gupta D A longitudinal study of steroid excretion patterns in children during adolescent growth *Steroidologia 1* 267 1970

11 Hammond G L Viinikka L & Vihko R Automation of radioimmunoassays for some sex steroids with use of both iodinated and tritiated ligands *Clin Chem 23* 1250 1977

1 Hammond G L Koivisto M Kouvalainen K & Vihko R Serum steroids and pituitary hormones in neonatal infants with particular reference to the decline in testicular activity in infant boys Submitted for publication 1978

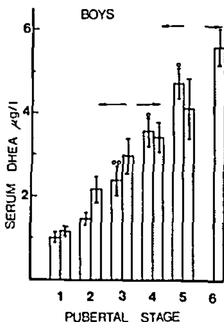


Fig 4 Serum DHEA according to external genitalia (light columns) and pubic hair (dark columns) stages in boys One to three of the symbols O or × indicate $p<0.05$ $p<0.01$ $p<0.001$ significance of differences in respect to the preceding stage or the stages marked by an arrow The bars indicate ±1 standard error of the mean The number of subjects in each group is 15–75 boys

crease until 18 5 years which was the oldest age group studied

Considerably less change is seen in the serum levels of ACTH and cortisol None of the changes seen in serum ACTH (Fig 1) are significant but there seems to be a slight decrease in serum ACTH concentration after 12 5 years of age Simultaneously serum cortisol increases (Table 1 Fig 1) suggesting a change in the regulation of the interaction of these two hormones When the girls are divided into premenarcheal and postmenarcheal groups serum cortisol is 92±35 (S D) µg/l ($n=115$) and 125±37 (S D) µg/l ($n=130$) respectively The latter value is significantly higher ($p<0.001$)

The corresponding data for the boys are shown in Fig 2 and Table 2 Serum DHEA also increases in the youngest boys but a period of a more rapid increase does not commence until after 12 5 years of age The increase continues without the appearance of a plateau over the age groups investigated

Serum cortisol decreases significantly ($p<0.01$) from 10 5 years of age until about 12 5 years of age After 16 5 years of age there is a similar relative change in serum ACTH and cortisol in boys as there is in girls (Figs 1 and 2) ACTH decreases while cortisol increases

A continuous increase is seen in serum DHEA in girls according to pubic hair and breast stages (Fig 3) The same is true in boys when they are grouped according to external genitalia and pubic hair stages (Fig 4) At the same pubic hair stages the girls have significantly higher concentrations of DHEA than boys ($p<0.01$) at each stage see Figs 3 and 4 The girls also show higher levels in each annual age group ($p<0.05$ or <0.01) with the exception of the oldest age group (Tables 1 and 2)

In both girls and boys serum cortisol correlates with DHEA ($p<0.001$) but not with ACTH

DISCUSSION

To obtain more information on adrenal function and its regulation in the course of puberty we investigated serum DHEA ACTH and cortisol in boys and girls 7–19 years of age

The effect of diurnal variation was minimized by always drawing blood samples at 08 00–10 00 a m To avoid stress effect on adrenal function no physical training was allowed on the morning of the investigation Also the nature of the study was carefully explained to the participants in advance to avoid psychological stress The large number of subjects investigated also diminishes the effect of stress on the final results

In accordance with earlier studies (7 8 14–17) we found that an increase in serum DHEA takes place very early In girls a significant increase was seen between the 7 5 and 8 5 year old groups according to bone age and an earlier increase cannot be ruled out Although slightly increasing values are also seen in the youngest boys the period of a more rapid increase appears later than in girls The importance of DHEA in the early physical signs

Acta Pædiatr Scand 68 605–607 1979

PEAK EXPIRATORY FLOW RATE

Reference Values for Swedish Children

JAN BJURE GUNNAR DALÉN and BENGT KJELLMAN

From the Department of Paediatric Clinical Physiology East Hospital Gothenburg and the Department of Paediatrics Karnsjukhuset Skovde Sweden

ABSTRACT Bjure J Dalen G and Kjellman B (Department of Paediatric Clinical Physiology East Hospital Gothenburg and Department of Paediatrics Karnsjukhuset Skovde Sweden) Peak expiratory flow rate Reference values for Swedish children Acta Paediatr Scand 68 605 1979 —Reference values for the peak expiratory flow rate assessed by the Wright–McKerrow peak flow meter have been established for Swedish children The material consisted of 143 boys and 132 girls We recommend the sexes be considered together The equation of the regression line is 72 14 × height^2 + 96 12 The coefficient of correlation is 0 93 and the residual standard deviation 13 7 [illegible]

KEY WORDS Healthy children peak expiratory flow rate

The peak expiratory flow meter (PEFM) described by Wright & McKerrow (7) is frequently used when examining children with pulmonary diseases Reference values for the peak expiratory flow rate (PEFR) assessed by the PEFM have been established for children by various authors (2 4 5) One of these studies covers Danish children but none Swedish children (2)

MATERIALS AND METHODS

Group A 11[illegible] boys and 10 girls selected from an ordinary school and investigated at the Department of Paediatric Clinical Physiology East Hospital Gothenburg

Group B 31 boys and 30 girls selected from an ordinary school and investigated at the Department of Paediatrics Karnsjukhuset Skovde

Table 1 presents the ages and heights of the children The principles of selection were the same for both groups and the same as in a previous study (1) All of the children were investigated in the sitting position One and the same PEFM (for adults) was used for the children of group A and an other PEFM (for adults) in group B Five or more good forced expiratory maneuvers were done by the children in group A and three in group B The highest values received in each case were used for the calculations

RESULTS AND COMMENTS

Group A had a small but significantly higher mean age ($p<0\ 05$) than group B whereas there were no significant differences concerning height and weight

The equations of the regression lines of

Table 1 *Two groups of healthy children investigated with the Wright peak flow meter*

Group	n	Age (years) Mean	Age (years) Range	Height (cm) Mean	Height (cm) Range
A boys	11	11 5	6 7–17 0	149	117–186
A girl	10	11 7	7 0–17 9	148	114–174
B boys	31	10 9	7 5–14 8	147	1[illegible]1–180
B girl	30	10 7	7 3–15 0	144	119–17

13 Kenny F M Adrenal function in adolescence *In* F P Heald & W Hung (eds) *Adolescent endocrinology* Appleton Century Crofts New York 1970 p 127
14 Korth Schutz S Levine L S & New M J Serum androgens in normal prepubertal and pubertal children and in children with precocious adrenarche *J Clin Endocrinol Metab* 42 117 1976
15 Parker L N Sack J Fisher D A & Odell W D The adrenarche Prolactin gonadotropins adrenal androgens and cortisol *J Clin Endocrinol Metab* 46 396 1978
16 Sizonenko P C & Paunier L Hormonal changes in puberty III Correlation of plasma dehydroepiandrosterone testosterone FSH and LH with stages of puberty and bone age in normal boys and girls and in patients with Addison s disease or hypogonadism or with premature or late adrenarche *J Clin Endocrinol Metab* 41 894 1975
17 Sizonenko P C Paunier L & Carmignac D Hormonal changes during puberty IV Longitudinal study of adrenal androgen secretions *Hormone Res* 7 288 1976
18 Tanner J M Growth and endocrinology of the adolescent *In* L J Gardner (ed) *Endocrine and genetic diseases of childhood and adolescence* W B Saunders Company Philadelphia London Toronto 1975 2nd ed p 14
19 Vermeulen A Suy E & Rubens R Effect of prolactin on plasma DHEA(S) levels *J Clin Endocrinol Metab* 44 1222 1977

Submitted Sept 27 1978
Accepted Dec 2 1978

(D A) Department of Medical Chemistry
University of Helsinki
Siltavuorenpenger 10 A
SF-00170 Helsinki 17
Finland

ACKNOWLEDGEMENTS

This investigation was supported by grants from For enade Liv Mutual Group Life Insurance Company Stockholm and Glaxo Lakemedel AB Goteborg Sweden Statistical aid by N G Pehrsson Datacentralen University of Gothenburg

REFERENCES

1 Dalen G & Kjellman B Evaluation of three spirometers on healthy children *Acta Paediatr Scand* In pre s 1978
Juhl B Pulmonary function investigations on 1011 school children using Wright s peak flow meter *Scand J Cl n Lab In est 25* 355 1970
3 Mantel N J *Am Stat Assoc Inst 2?* 719 1963
4 Murray A B & Cook C D Measurement of peak expiratory flow in _0 normal children from 4 5 to 18 5 years of age *J Pediatr 6?* 186 1963
5 Nairn J R Bennet A J Andrew J D & MacArthur P A study of respiratory function in normal school children The peak flow rate *Arch Dis Child 36* _53 1961
6 Oden A & Wedel H Arguments for Fishers permutation test *Ann Stat 3* 518 1975
7 Wright B M & McKerrow C B Maximum forced expiratory flow as a measure of ventilatory capacity With discription of a new portable instrument for measuring it *Br Med J II* 1041 1959

Submitted Oct 13 1978
Accepted Dec 3 1978

(G D) Department of Paediatrics
Karnsjukhuset
S 54101 Skovde
Sweden

Table 2. *Equations of the regression lines of peak expiratory flow rate (PEFR l/min) to height in metre raised to the third power (H^3) and coefficients of correlation (r) for two groups of healthy children*

Residual standard deviations around the lines (R.S.D.) are also given as l/min and as percentages of the mean values

Group	Equation of the line PEFR =	r	R.S.D. (l/min)	R.S.D. (%)
A total	$71.95 \times H^3 + 101.99$	0.93	46.0	13.2
A boys	$68.89 \times H^3 + 110.79$	0.94	45.7	13.1
A girls	$77.11 \times H^3 + 86.51$	0.93	46.3	13.4
B total	$67.70 \times H^3 + 91.86$	0.91	42.7	14.0
B boys	$69.80 \times H^3 + 90.39$	0.92	46.5	14.6
B girls	$61.42 \times H^3 + 105.47$	0.88	38.9	13.3
A+B total	$72.14 \times H^3 + 96.12$	0.93	46.2	13.7

PEFR with respect to height (raised to the third power) are given in Table 2. To test if the PEFR values of group A differed significantly from the values of group B for a given height and sex a nonparametric partial analysis was performed. The groups were divided in a number of intervals with respect to sex and height. Within each such interval Fishers permutation test variable was determined (6). The test variables from the different intervals of height were pooled (3). The values of the total group A (boys and girls) and that of the girls of group A were found to be significantly higher ($p<0.01$) than the corresponding values of group B. *No significant difference was found* for the boys of the two groups.

PEFR values for three heights calculated from the equations are given in Table 3. Corresponding values given by three other studies are also shown for comparison.

The correlation coefficients and residual standard deviations (R.S.D.) in our study are similar to those found in other studies (2, 4, 5).

The variations between different studies have several possible causes, e.g. differences of race, of selection and composition of the groups, of the procedure used and the cooperation of the subject and differences between the various PEFM used. As the differences between groups A and B are small compared to the great R.S.D. and the uncertain factors we consider it suitable to combine our two groups and recommend the sexes be considered together as Nairn et al. (5). As reference data for Swedish children we recommend total groups A and B (Table 3).

Table 3. *Predicted mean peak expiratory flow rate (PEFR) of healthy children for the heights 130 cm, 145 cm and 160 cm*

The results of four studies are given. R.S.D. = residual standard deviations around the lines as percentages of the mean values

Investigation	n	R.S.D. (%) ♂	R.S.D. (%) ♀	PEFR at three heights: 130 cm ♂	130 cm ♀	145 cm ♂	145 cm ♀	160 cm ♂	160 cm ♀
Present Group A	214	13.1	13.4	262	256	321	322	393	402
Present group B	61	14.6	13.3	244	240	303	293	376	357
Present groups A + B	275	13.7		255		316		392	
Juhl (1970)	779	12.9	12.2	242	237	321	311	399	385
Murray & Cook (1963)	220	14.5	14.3	261	261	347	330	432	400
Nairn et al. (1961)	471	13.4		251	282	335	362	418	442

Acta Pædiatr Scand 68 609–612 1979

HEMOGLOBIN A_1 (HbA_{1c}) IN CHILDREN WITH LONG STANDING AND NEWLY DIAGNOSED DIABETES MELLITUS

E HEINZE E KOHNE C MEISSNER W BEISCHER
W M TELLER and E KLEIHAUER

From the Department of Paediatrics University of Ulm/Donau F R G

ABSTRACT Heinze E Kohne E Meissner C Beischer W Teller W M and Kleihauer E (Department of Pediatrics University of Ulm/Donau F R G) Hemoglobin A (HbA) in children with long standing and newly diagnosed diabetes mellitus Acta Paediatr Scand 68 609 1979 —In 35 children with long standing diabetes mellitus a significant correlation was found between the hemoglobin A (HbA)—and the 24 hour urinary glucose excretion By contrast 11 newly diagnosed diabetic children had grossly elevated HbA concentrations but no correlations could be established between the levels of HbA and the duration of symptoms blood glucose glycosuria ketonuria and the acid–base status However HbA and C peptide were significantly correlated The elevated HbA -concentrations decreased towards normal in all of these 11 children after 2–3 months following adequate therapy The results suggest that the determination of HbA may serve as a valuable metabolic control index in children with long standing diabetes mellitus but adds little information in newly diagnosed diabetic patients For the individual diabetic child during the early treatment period HbA may be the index of choice for adequacy of metabolic control

KEY WORDS Diabetes mellitus children HbA metabolic control

Rabhar Blumenfeld & Ramney (16) were the first to report an elevation of hemoglobin A_1 (HbA_1) in diabetic patients This was confirmed by others and in addition a close correlation between the metabolic control of the disease and the elevation of HbA_1 seemed established (4 8 12 13 14)

In the present study HbA_1 was determined in two divided groups of diabetic children in one group were children with diabetes of more than one year s duration in the other group HbA_1 was measured sequentially from the time of diagnosis at various time intervals following the onset of treatment

MATERIAL AND METHODS

Patients Forty six diabetic children aged 1 5 to 18 years were studied According to the duration of their disease they were divided into two groups (repetition see above) ...ts with diabetes over one year s duration (range 1–1? years) Group two consisted of 11 diabetic children who were regularly studied from the time of diagnosis and throughout the following months of therapy Pertinent clinical and laboratory data are presented in Table 1

All 46 children received a diet which contained 40–45% of calories as carbohydrate 35–40% fat and 15–20% protein Insulin replacement was achieved with a mixture of a regular and an intermediate insulin preparation twice a day The amount of insulin was adjusted according to the urinary glucose excretion which was tested at home three times a day by Clinitest® All patients were regularly followed in the outpatient clinic A 24-hour urine was collected at home utilizing random samplings i e in the morning the afternoon and the night

Methods Blood glucose was measured with a hexokinase method (16) the capillary pH and the P by routine analysis the urine sugar polarimetrically and the aceton excretion with Acetest® The immunoreactive C peptide was determined as previously described (1) HbA was measured by column chromatography according to Huisman & Meyering (10) with minor modifications (11)

The correlation coefficient was calculated with a Hewlett Packard calculator 9810A connected with a Hewlett Packard calculator plotter 98 6A to draw the regression line automatically

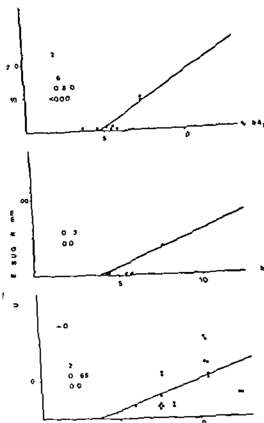


Fig 1 Correlations between HbA and the urinary glucose in diabetic children with long standing diabetes mellitus at two months (-2) one month (-1) prior and at the time (0) of the Hb determination

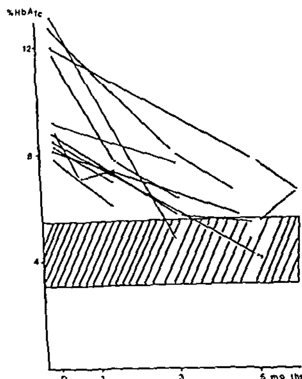


Fig 2 Pattern of the HbA -concentration in 11 diabetic children from the time of diagnosis and during the following months The shaded area indicated the normal range

(3 8) glucagon free insulin growth hormone (8) and the thickness of the basement membrane (12) There was some degree of correlation in some cases between HbA_1 concentrations and cholesterol as well as triglyceride but none was found between the former and hormones or changes of the basement membrane

No significant correlations were forthcoming beween the HbA_1 levels and the duration of symptoms the blood and urine glucose concentrations the acid-base status or the ketone body excretion in the present study of the 11 newly diagnosed diabetic children However the positive correlation between the C peptide and the HbA_1 concentrations was unexpected

Very recently highly specific insulin receptors have been identified on human erythrocytes There are as yet few but controversial findings relating to the physiological role of insulin on the glucose transport system of human red cells (7) On the other hand since insulin is released in equimolar amounts with C peptide from the B cells it may well be that insulin modulates the intracellular glucose concentration in the human red cells No correlation was found between the blood glucose and the C peptide concentration which underscores the insensitivity of the B cells in juvenile diabetics to glucose (9)

It should be emphasized that all determinations were performed in the non fasting state which could explain the discrepancies between our data and those of a recent study in newly diagnosed juvenile diabetics (3) There serum indices were determined in the

Table 1 *Clinical and laboratory data of 11 newly diagnosed diabetic children on admission*

Case	Sex	Age (years)	Onset-weeks	Blood glucose (mg/dl)	pH	Base exc (mval/l)	Urine sugar (mmol/l)	Acetest[a]	C Pep (ng/ml)	HbA_1 (%)
1	M	10 5	6	223	7 26	−9	361	++	0 7	9 2
2	F	12 5	2	225	–	–	600	+++	0 8	8 8
3	M	14 5	1	1 167	6 95	−24	416	+++	<0 5	8 9
4	F	11 5	5	240	–	–	222	+	0 8	7 8
5	F	1 5	6	250	7 38	−4	194	++	<0 5	8 3
6	M	8	4	370	7 04	−24	278	+++	0 9	12 0
7	F	13 5	20	223	7 46	+2	378	+	2 0	11 8
8	F	3 2	3	235	–	–	355	++	0 8	13 1
9	M	10 5	0 5	460	7 38	−4	539	+++	0 7	8 4
10	M	9 2	3	225	–	–	417	++	<0 5	6 2
11	F	11 5	8	430	7 35	−6	522	++	2 2	12 7
r^a			0 392	0 208	0 155	0 712	0 036	0 022	0 656	
p			n s	n s	n s	n s	n s	n s	<0 05	

r = correlation coefficients versus HbA_1

RESULTS

Fig 1 shows the results of the patients from group one of more than one year s duration The urine sugar concentrations mmol/l represent the mean of the three separately collected specimens Hemoglobin A_{1c} (HbA_{1c}) was measured and the regression analyses were plotted against the urine glucose concentrations simultaneously with (0 months) and one month (−1 month) and two months (−2 months) prior to the determination of the glycolysated hemoglobin A A significant correlation was found between the urine glucose concentrations and HbA_{1c}

In group two the correlation coefficients were calculated between HbA_{1c} concentrations and the following parameters the time when the first symptoms suggestive of diabetes mellitus were noted by the patients the initial blood glucose concentrations the pH the base excess the urine glucose the acetone excretion and the immunoreactive serum C peptide concentrations (Table 1) A correlation of statistical significance was only found between the HbA_{1c} and the C peptide concentrations while the correlation coefficient between the C peptide and the blood glucose levels was $r=0$ 148

During the following months when the 11 diabetic children were treated effectively with diet and insulin the grossly elevated HbA_{1c} concentrations declined in all patients and reached the normal range in 3 children Fig 2

DISCUSSION

During recent years numerous authors could show that the elevated HbA_{1c} concentrations correlated very well with the blood and the urinary glucose concentrations in juvenile and adult onset diabetes (4 8 12 13 14)

In the present study the results obtained in the 35 children who suffered from diabetes mellitus for more than one year confirm the previous reports Furthermore Gabbay et al s (6) found the highest correlation between the glycosylated hemoglobin and the amount of glucose excretion in the specimens collected 2 months prior to the determination of HbA_{1c} It was suggested that the overwhelming formation of HbA_{1c} predominantly occurs during the first half of the 120 days life span of the red cells The observation that a plateau of the HbA_{1c} concentration was reached in 60- to 80 day old erythrocytes tends to support this conclusion (2)

To further characterize the metabolic profile in the diabetic patients correlations were looked for between the glycolysated hemoglobin and cholesterol (3 6 8) triglycerides

LETTER TO THE EDITOR

PLASMA PREALBUMIN IN THE NEWBORN

Sir

We read with interest the paper of Jacobsen and associates (1) The authors emphasize that in new born infants with birth weights appropriate for gestation serum prealbumin concentrations increase progressively with gestational age and that in small for gestational age babies the levels are significantly lower Recently we reported on the same subject and could also find that in pre term infants plasma prealbumin at birth increases with foetal age (3) However only one out of nine small for dates new born infants had a plasma prealbumin level below the normal range Also our recent data shown in Table 1 and presented according to the findings of Jacobsen et al (1) do not suggest that plasma prealbumin is a reliable index of foetal malnutrition As plasma levels in control male adults 29 7 (1) versus 27 7 (3) mg/dl are similar in both studies the apparent discrepancy can hardly be explained by technical differences From the data presented in both studies it seems also unlikely that the observed discrepancy is due to differences in sampling time first postnatal week (1) and at birth only (3) However it is noteworthy that only our study compares appropriate for dates and small for dates pre term infants and that the statistical analysis of our data shows a high variance in the group of small for dates full term infants which suggests high individual differences in the study group

Table 1 *Plasma prealbumin (mg/dl) in the newborn*

	Pre term infants (≤36 weeks)		Full term infants (37–41 weeks)	
	AGA	SGA	AGA	SGF
Number	64	10	76	8
Mean	8 10	7	1[illegible] 5	10 5
SEM	0 43	0 93	0 69	1 93
Variance	1[illegible]	8 7	1[illegible] 40	[illegible]9 70

Appropriate for gestational age newborns (AGA) have birth weights between P and P_{90} for foetal age small for gestational age infants (SGA) have birth weights below P for foetal age (2) There is no statistical difference between AGA and SGA pre term infants or between AGA and SGA full term infants Statistical differences ($p < 0.001$) are found only between AGA pre term and AGA full term newborns and between SGA pre term and AGA full term babies

E Eggermont S Bhavani
J Socha H Carchon

Department of Paediatrics
Academisch Ziekenhuis Gasthuisberg
Leuven
Belgium

REFERENCES

1 Jacobsen B B Pettersen B Andersen H J & Hummer L Serum concentrations of thyroxine binding globulin prealbumin and albumin in healthy fullterm small for gestational age and preterm new born infants *Acta Paediat Scand* 68 49 1979
2 Lubchenco L O *The high risk infant* Saunders Company Philadelphia 1976 1st ed p 65
3 Socha J Eggermont E Carchon H Devlieger H & Eeckels R Plasma prealbumin in low birthweight infants *Acta Paediatr Belg* 30 171 1977

fasting state and significant correlations were found between glucose, cholesterol, triglyceride on the one hand and the HbA_{1c} concentration on the other.

Our results do suggest, however, that determinations of HbA_{1c} give no further information about the acute metabolic situation in newly diagnosed *juvenile onset diabetes*. Furthermore, assuming that the glycolysation of hemoglobin A in diabetics occurs under similar conditions as in normals with a plateau of HbA_{1c} concentration in 60–80-day old red cells, it appears that a considerable degree of hyperglycemia may be tolerated without any clinical symptoms suggestive of diabetes mellitus (2, 5).

During remission induced by appropriate treatment, the elevated HbA_{1c} concentrations declined in all 11 recent onset diabetics. This suggests that the glycosylated hemoglobin is an excellent indicator for the metabolic control in the individual patient during the remission phase of juvenile onset diabetes mellitus.

ACKNOWLEDGEMENTS

We deeply thank Professor J. R. Poley, University of Virginia, USA, who corrected the style and the language of the manuscript. Furthermore, the expert technical assistance of Miss Maria Maas and Miss M. Krause and the secretarial help of Mrs E. Kraus is gratefully acknowledged.

REFERENCES

1. Beischer W, Keller L, Maas M, Schiefer E & Pfeiffer E F. Human C-peptide. Part I. Radioimmuno assay. *Klin Wochenschr 54*: 709, 1976.
2. Bunn H F, Haney D N, Kamin S, Gabbay K H & Gallop P M. The biosynthesis of human hemoglobin A_1. Slow glycosylation of hemoglobin in vivo. *J Clin Invest 57*: 1652, 1976.
3. Ditzel J & Kjaergaard J J. Hemoglobin A_1 concentration for newly discovered diabetes. *Br Med J I*: 741, 1978.
4. Dolhofer R, Stadele A & Wieland O H. Clinical and biochemical studies on the significance and formation of hemoglobin A_1 and A_{1a+b} in diabetes mellitus. *Klin Wochenschr 55*: 945, 1977.
5. Fitzgibbons J F, Koler R D & Jones R T. Red cell age related changes in hemoglobins A_{1a+b} and A_{1c} in normal and diabetic subjects. *J Clin Invest 58*: 820, 1976.
6. Gabbay K H, Hasty K, Breslow J L, Ellison R C, Bunn H F & Gallop P M. Glycosylated hemoglobins and long term blood glucose control in diabetes mellitus. *J Clin Endocrinol Metab 44*: 859, 1977.
7. Gambhir K K, Archer J A & Bradley D J. Characteristics of human erythrocyte insulin receptors. *Diabetes 27*: 701, 1978.
8. Gonen B, Rochman H, Rubenstein A H, Tanega S P & Horwitz D L. Haemoglobin A_1: An indicator of the metabolic control of diabetic patients. *Lancet II*: 734, 1977.
9. Heinze E, Beischer W, Keller L, Winkler G, Teller W M & Pfeiffer E F. C-peptide secretion during the remission phase of juvenile diabetes. *Diabetes 27*: 670, 1978.
10. Huisman T H J & Meyering C H. Studies on the heterogenity of hemoglobin. I. The heterogeneity of different human hemoglobin types in carboxymethylcellulose and in Amberlite IRC 50 chromatography. Quantitative aspects. *Clin Chim Acta 5*: 103, 1960.
11. Huisman T H & Jonxis J H P. The hemoglobinopathies. Techniques of identification. *Clinical and biochemical analysis*. Marcel Dekker Inc. New York and Basel 1977, vol. 6, pp. 126–131.
12. Koenig R J, Peterson C M, Kilo C, Cerami A & Williamson J R. Hemoglobin A_{1c} as an indicator of the degree of glucose intolerance in diabetes. *Diabetes 25*: 230, 1976.
13. Lanoe R, Thibult N, Eschwege E, Soria J, Soria C & Tchobrontsky G. Glycosylated haemoglobin concentrations and Clinitest results in insulin-dependent diabetes. *Lancet II*: 1156, 1977.
14. Paulsen E P. Hemoglobin A_1 in childhood diabetes. *Metabolism 22*: 269, 1973.
15. Rahbar S, Blumenfeld O & Ranney H M. Studies of an unusual hemoglobin in patients with diabetes mellitus. *Biochem Biophys Res Commun 36*: 838, 1969.
16. Schmidt F H. Enzymatische Methode zur Bestimmung von Blut- und Harnzucker unter Berücksichtigung von Vergleichsuntersuchungen mit klassischen Methoden. *Internist 4*: 554, 1963.

Submitted Sept. 20, 1978
Accepted Jan. 20, 1979

(E. H.) Department of Pediatrics
University of Ulm
Prittwitzstrasse 43
D-7900 Ulm (Donau)
F.R.G.

Acta Pædiatr Scand 68 615–616 1979

LETTER TO THE EDITOR

Sir

Although the mechanism proposed by Dr Katznelson (1) for the increased intracranial pressure observed in three infants with cystic fibrosis cannot be discounted another explanation is possible It has been reported previously (2) that a *deficiency* of vitamin A may predispose to development of increased intracranial pressure and bulging fontanelles in cystic fibrosis In fact we have observed a similar syndrome in infants who have been malnourished prior to the diagnosis of cystic fibrosis and who do not have severe respiratory distress Dr Katznelson does not mention whether his three patients were treated with enzymes and/or vitamins during their hospital course If so this could account perhaps for the decrease in intracranial pressure noted

Finally it is somewhat difficult to understand how such marked airway obstruction which produced increased intracranial pressure could occur with preservation of arterial blood gases The relatively high $P_{a_{O_2}}$ and appropriately low $P_{a_{CO_2}}$ in cases 1 and 2 leads one to believe that the airway obstruction could not have been excessive at least in these two patients

Lynn M Taussig
Associate Professor of Pediatrics

Arizona Health Sciences Center
Tucson Arizona 857 4
USA

REFERENCES

1 Katznelson D Increased intracranial pressure in cystic fibrosis *Acta Paed atr Scand 67* 607 1978
2 Keating J P & Feigin R D Increased intracranial pressure associated with probable Vitamin A deficiency in cystic fibrosis *Ped atrics 46* 41 1970

The Editor has asked Dr Katznelson to comment on this letter

I want to thank Dr Taussig for drawing attention to deficiency of vitamin A as yet another cause for increased intracranial pressure Vitamin A and carotene blood levels were not determined in our cases and therefore obviously this possibility cannot be excluded at least in two of the cases Case 2 had a bulging fontanel at the age of one month This is possibly too short a period in which to develop significant avitaminosis in our infant who had been perfectly normal nutritionally at birth In the paper referred to by Dr Taussig (1) both infants had had overt clinical signs of vitamin A deficiency None of our cases had had xeropthalmia

Dr Taussig s suggestion that the improvement in the intracranial pressure in our cases was really the result of the unknowing associated administration of vitamin A and not to the improvement in the pulmonary status as I proposed fails to note the clinical observation that there had been a rise and fall in the bulge of the fontanel concomitantly with the increase and decrease of respiratory obstruction signs This cycle occurred twice in Case 2 and three times in Case 3 and all in the space of a few days Had the first episode of bulging fontanel been due to avitaminosis A and its alleviation the result of therapy then yet another mechanism must be marshalled to explain the subsequent episodes of recurring bulge of the fontanel

As to the question of the severity of airway obstruction the clinical reversible picture of severe tachypnea with poor air entry clear lung fields on X ray with overdistended lungs and small heart appear most suggestive of air

Acta Pædiatr Scand 68 617–618 1979

SHORT COMMUNICATION

PLASMA LEVELS OF COMPLEMENT FACTORS 3 AND 4 OROSOMUCOID AND OPSONIC FUNCTIONS IN ANOREXIA NERVOSA

Previous studies have shown that various alterations of the defence against infectious agents can occur in anorexia nervosa patients (6 10) In a case report Kim & Michael (5) described changes in the serum complement system including depression of C3 while C4 remained normal Since nothing is known about the frequency of alterations in the complement system and other acute phase reactants in anorexia nervosa 10 consecutive patients have been studied with regard to the plasma levels of complement factors 3 and 4 (C3 & C4) orosomucoid and the opsonic functions of plasma for PMN granulocytes

The anorexia nervosa patients 9 girls and one boy (for details see refs 3 10) conformed to previously described criteria for the disease (3) The mean age was 14 7 years and the mean weight loss was 27% from premorbid weight All patients but one were below −2 S D relating weight to height Fifteen normal weight controls were investigated simultaneously Details concerning them have been published previously (10)

Heparinized plasma samples from the patients and controls were frozen at −50°C within 2 hours of withdrawal and thawed only just prior to assay C3 C4 and orosomucoid levels were determined by an electro-immunoassay (7 cf 11)

The opsonic function of plasma was analysed with an assay for PMN bactericidal activity (9) PMNs from healthy donors were incubated with *Staph aureus* and 1% plasma for 90 min The results are expressed as the percentages living CFU of the initial CFU counts

The anorexia patients had a significantly lower mean value ($p<0.001$) for their plasma C3 levels than the controls (Fig 1) Five of the 10 patients showed values outside the reference values (mean −2 S D) No significant differences between patients and controls were found for C4 and orosomucoid levels and opsonic functions of plasma (Fig 1) Nor were any significant correlations found in the anorexia patients between on the one hand values for the C3 C4 and orosomucoid levels and on the other for weight weight loss from premorbid weight and opsonic functions (Student s *t* test and linear regression)

The present C3 decreases could be due to a diminished synthesis changes in the distribu

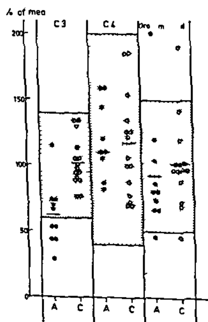


Fig 1 Plasma C3 C4 and orosomucoid concentrations in anorexia nervosa patients (A ●) and in normal weight controls (C ○) Mean value — The shaded area represents the reference values (means ±2 S D) The opsonic function test (mean values + S E) anorexia patients 8 9±0 6% living CFU controls 7 4±0 6% (n s)

way obstruction in C F In Case 3 the blood gas levels are in keeping with this In Case 1 the P_{aCO_2} was only mildly low (P_{aCO_2} 4 7 kPa) It is only in Case 2 that the P_{aCO_2} was very low (P_{aCO_2} 3 1 kPa) This of course must have been the result of the hyperventilation itself indicative of the degree of respiratory embarrassment and refers to the time of admission and not to the situation three days later when the fontanel bulged It is not impossible that the P_{aCO_2} may have been higher

D Katznelson

Dept of Pediatrics A
The Chaim Sheba Medical Center
Tel Hashomer
Israel

Acta Pædiatr Scand 68 617–618 1979

SHORT COMMUNICATION

PLASMA LEVELS OF COMPLEMENT FACTORS 3 AND 4 OROSOMUCOID AND OPSONIC FUNCTIONS IN ANOREXIA NERVOSA

Previous studies have shown that various alterations of the defence against infectious agents can occur in anorexia nervosa patients (6 10) In a case report Kim & Michael (5) described changes in the serum complement system including depression of C3 while C4 remained normal Since nothing is known about the frequency of alterations in the complement system and other acute phase reactants in anorexia nervosa 10 consecutive patients have been studied with regard to the plasma levels of complement factors 3 and 4 (C3 & C4) orosomucoid and the opsonic functions of plasma for PMN granulocytes

The anorexia nervosa patients 9 girls and one boy (for details see refs 3 10) conformed to previously described criteria for the disease (3) The mean age was 14 7 years and the mean weight loss was 27% from premorbid weight All patients but one were below −2 S D relating weight to height Fifteen normal weight controls were investigated simultaneously Details concerning them have been published previously (10)

Heparinized plasma samples from the patients and controls were frozen at −50°C within 2 hours of withdrawal and thawed only just prior to assay C3 C4 and orosomucoid levels were determined by an electro-immunoassay (7 cf 11)

The opsonic function of plasma was analysed with an assay for PMN bactericidal activity (9) PMNs from healthy donors were incubated with *Staph aureus* and 1% plasma for 90 min The results are expressed as the percentages living CFU of the initial CFU counts

The anorexia patients had a significantly lower mean value ($p<0.001$) for their plasma C3 levels than the controls (Fig 1) Five of the 10 patients showed values outside the reference values (mean −2 S D) No significant differences between patients and controls were found for C4 and orosomucoid levels and opsonic functions of plasma (Fig 1) Nor were any significant correlations found in the anorexia patients between on the one hand values for the C3 C4 and orosomucoid levels and on the other for weight weight loss from premorbid weight and opsonic functions (Student s *t* test and linear regression)

The present C3 decreases could be due to a diminished synthesis changes in the distribu

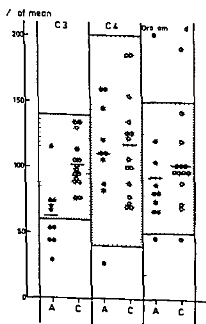


Fig 1 Plasma C3 C4 and orosomucoid concentrations in anorexia nervosa patients (A ●) and in normal weight controls (C O) Mean value — The shaded area represents the reference values (means ±2 S D) The opsonic function test (mean values + S E) anorexia patients 8 9±0 6% living CFU controls 7 4±0 6% (n s)

tion between different compartments or increased catabolism. The data in the present study do not indicate which mechanism(s) might be responsible. Although decreased synthesis has usually been suggested as the mechanism responsible for low C3 levels in studies on undernourished patients from developing countries, these have also shown signs of an increased C3 consumption and increased levels of other acute phase reactants, probably secondary to coexisting infections (2, 8, 13). In this study, however, normal C4 and orosomucoid levels together with absence of clinical signs of infections or other diseases apart from the anorexia nervosa, make explanations based on a complement activation by the classical or alternative pathway less likely. Hence, a diminished synthesis is a probable explanation for the low C3 levels (cf. 5).

Decreased serum levels of acute phase reactants have been reported in undernourished subjects and in fasting (8, 9, 11). The anorexia patients showed normal orosomucoid levels, however, which could be due to the absence of coexisting infections and the fact that protein depletion is of minor importance in this disorder compared with other forms of undernutrition (1).

The finding of a normal opsonic function of 1% plasma despite low C3 levels also agrees with previous studies on chronically undernourished subjects and findings in total fasting (9, 12, cf. however 4), where opsonic functions have most often been normal.

It is concluded that in anorexia nervosa low C3 levels together with decreased granulocyte functions (10) might contribute to a decreased resistance to e.g. bacterial infections.

J Palmblad *Lars Fohlin* *Renee Norberg*

Department of Medicine IV
Karolinska Institutet
Sodersjukhuset
S 100 64 Stockholm 38
Sweden

REFERENCES

1. Aperia A, Broberger O & Fohlin L. Renal function in anorexia nervosa. *Acta Paediatr Scand* 67: 219, 1978.
2. Chandra R K. Serum complement components in malnourished Indian children. *In* R M Suskind (ed.): *Malnutrition and the Immune Response*. Raven Press, New York 1977, p. 329.
3. Fohlin L. Body composition, cardiovascular and renal function in adolescent patients with anorexia nervosa. *Acta Paediatr Scand* 66, Suppl 268, 1977.
4. Keusch G T, Urrutia J J, Fernandez R, Guerrero O & Casteneda G. Humoral and cellular aspects of intracellular bacterial killing in Guatemalan children with protein calorie malnutrition. *In* R M Suskind (ed.): *Malnutrition and the Immune Response*. Raven Press, New York 1977, p. 245.
5. Kim Y & Michael A F. Hypocomplementemia in anorexia nervosa. *J Pediatr* 87: 582, 1975.
6. Kjosen B, Bassoe H H & Myking O. The glucose oxidation in isolated leukocytes from female patients suffering from overweight or anorexia nervosa. *Scand J Clin Lab Invest* 35: 447, 1975.
7. Laurell C B. Electroimmunoassay. *Scand J Clin Lab Invest* 29, Suppl 124: 21, 1972.
8. McFarlane H. Acute phase proteins in malnutrition. *In* R M Suskind (ed.): *Malnutrition and the Immune Response*. Raven Press, New York 1977, p. 403.
9. Palmblad J. Fasting (acute energy deprivation) in man: effect on polymorphonuclear granulocyte functions, plasma iron and serum transferrin. *Scand J Hematol* 17: 217, 1976.
10. Palmblad J, Fohlin L & Lundstrom M. Anorexia nervosa and polymorphonuclear (PMN) granulocyte reactions. *Scand J Hematol* 19: 334, 1977.
11. Palmblad J, Cantell K, Holm G, Norberg R, Strander H & Sundblad L. Acute energy deprivation in man: effect on serum immunoglobulins, antibody response, complement factors 3 & 4, acute phase reactants and interferon producing capacity of blood lymphocytes. *Clin Exp Immunol* 30: 50, 1977.
12. Seth V & Chandra R K. Opsonic activity, phagocytosis and bactericidal capacity of polymorphs in undernutrition. *Arch Dis Child* 47: 282, 1972.
13. Sirisinha S, Suskind R M, Edelman R, Kulapongs P & Olson R E. The complement system in protein calorie malnutrition—a review. *In* R M Suskind (ed.): *Malnutrition and the Immune Response*. Raven Press, New York 1977, p. 309.

Acta Pædiatr Scand 68 619–620 1979

SHORT COMMUNICATION

HEREDITARY TYROSINAEMIA AND DIABETES MELLITUS

The main features in hereditary tyrosinaemia are liver damage leading to cirrhosis and renal tubular defects with hypophosphataemic rickets (4) Pain attacks in the abdomen and legs similar to those seen in acute intermittent porphyria and hypertensive crisis have been reported in this disorder (4) We report here a boy with similar symptoms and signs but who also had diabetes mellitus

Patient

Boy first child to healthy unrelated parents no heredity for diabetes mellitus Birth weight 3 180 g After weaning at 3 months he vomited and had a peculiar odour Admitted at 6 months because of fever and abdominal pain He had hepato-splenomegaly and foeter hepaticus Reduced liver produced coagulation factors (e g P & P 5%) hypophosphataemia (0 8 mmol/l) glucosuria generalized aminoaciduria with high excretion of methionine tyrosine and phenolic acids increased urinary excretion of δ aminolevulic acid porphobilinogen in urine negative P tyrosine and P methionine increased (Table 1) Phenylalanine tolerance test (100 mg/kg body weight) gave a maximum value of P tyrosine of 883 µmol/l at 5 h and a slow decrease to 552 µmol/l at 24 h Correspondingly an increased urinary excretion of phenolic acids was observed Fructose tolerance test (50 mg/kg) was normal P-ceruloplasmin and P-orosomucoid levels were remarkably low and P albumin moderately decreased (Table 1) Skeletal X ray showed no signs of rickets He was treated with a tyrosine and phenylalanine restricted diet supplemented with iron ascorbic acid vitamin D and other vitamins Marked clinical improvement with increasing levels of coagulation factors (e g P & P 24%) normophosphataemia and reduced levels of P tyrosine but P-ceruloplasmin and P-orosomucoid were still low (Table 1) From 15 months hypophosphataemia increasing urinary volume no glucosuria At 19 months increasing thirst and large urine volume hyperglycaemia (45 mmol/l) glucosuria but no keto acidosis P-insulin 3 µE/ml The same low level of P-ceruloplasmin and P-orosomucoid otherwise normal plasma protein electrophoresis (Table 1) Insulin-dependent diabetes mellitus was diagnosed requiring 12 U insulin per day The diet was modified to satisfy as far as possible the demands of the two diseases During the next year he was in a remarkably good condition and had a normal motoric and mental development his weight was 1 5 kg and height 87 cm at age 5 years At 2½ years he had his first hypokalaemic crisis (2 9 mmol/l) with acidosis At 3 years pain in abdomen and legs keto acidosis and hypokalaemia blood pressure 130/100 mmHg Urinary volume 3–4 l/day Insulin requirement between 10 and 20 U per day P-ceruloplasmin and P orosomucoid decreased (Table 1) During the next three years he had 12 similar attacks with pains predominantly in the legs hypokalaemia (lowest value 1 4 mmol/l) hypophosphataemia renal acidosis and urinary volume up to 7 l/day At four years radiological signs of rickets and also some pathological fractures Increasing muscle wastage and inability to walk Supplementary large doses of potassium vitamin D phosphate and bicarbonate were tried orally At 6 years he also had a hypertensive crisis blood pressure 160/120 mmHg and cerebral symptoms Increased levels of P aldosterone P renin and increased urinary excretion of aldosterone but not of catecholamines At 6¾ years he had a hypertensive crisis 230/150 mmHg with encephalopathy and increased urinary excretion of aldosterone and catecholamines he died in a picture of cardiac failure Autopsy was made 48 h after death Advanced autolytic changes made a detailed microscopical investigation impossible The main gross lesions were a nodular cirrhosis of the liver a hypertrophy of the left side of the heart and slight signs of cardiac failure with a moderate pulmonary edema and marked congestion of the liver and the spleen As far as could be seen the islets of Langerhans and the suprarenal glands were normal Some crystalline granules were found in the renal tubular epithelium (Autopsy performed by Dr I Glifberg Department of Pathology Malmö General Hospital Malmö)

This patient fulfils the diagnostic criteria for hereditary tyrosinaemia of the acute type (7) He also had hypertensive crisis intermittent pains and increased levels of P aldosterone and P renin The mechanism for these abnormalities will be discussed more fully elsewhere (to be published)

The isolated decrease of P-ceruloplasmin and P orosomucoid has not been reported earlier in this disorder The cause of this abnormality is obscure

Diabetes mellitus has not been reported before in hereditary tyrosinaemia Several of the biochemical abnormalities in hereditary tyrosinaemia such as hypokalaemia acidosis hypophosphataemia and hypermethionae

tion between different compartments or increased catabolism The data in the present study do not indicate which mechanism(s) might be responsible Although decreased synthesis has usually been suggested as the mechanism responsible for low C3 levels in studies on undernourished patients from developing countries, these have also shown signs of an increased C3 consumption and increased levels of other acute phase reactants probably secondary to coexisting infections (2 8 13) In this study however normal C4 and orosomucoid levels together with absence of clinical signs of infections or other diseases apart from the anorexia nervosa make explanations based on a complement activation by the classical or alternative pathway less likely Hence a diminished synthesis is a probable explanation for the low C3 levels (cf 5)

Decreased serum levels of acute phase reactants have been reported in undernourished subjects and in fasting (8 9 11) The anorexia patients showed normal orosomucoid levels however which could be due to the absence of coexisting infections and the fact that protein depletion is of minor importance in this disorder compared with other forms of undernutrition (1)

The finding of a normal opsonic function of 1% plasma despite low C3 levels also agrees with previous studies on chronically undernourished subjects and findings in total fasting (9 12 cf however 4) where opsonic functions have most often been normal

It is concluded that in anorexia nervosa low C3 levels together with decreased granulocyte functions (10) might contribute to a decreased resistance to e g bacterial infections

J Palmblad　*Lars Fohlin*　*Renee Norberg*

Department of Medicine IV
Karolinska Institutet
Sodersjukhuset
S 100 64 Stockholm 38
Sweden

REFERENCES

1 Aperia A Broberger O & Fohlin L Renal function in anorexia nervosa *Acta Paediatr Scand 67* 219 1978
2 Chandra R K Serum complement components in malnourished Indian children *In* R M Suskind (ed) *Malnutrition and the Immune Response* Raven Press New York 1977 p 329
3 Fohlin L Body composition cardiovascular and renal function in adolescent patients with anorexia nervosa *Acta Paediatr Scand 66* Suppl 268 1977
4 Keusch G T Urrutia J J Fernandez R Guerrero O & Casteneda G Humoral and cellular aspects of intracellular bacterial killing in Guatemalan children with protein calorie malnutrition *In* R M Suskind (ed) *Malnutrition and the Immune Response* Raven Press New York 1977 p 245
5 Kim Y & Michael A F Hypocomplementemia in anorexia nervosa *J Pediatr 87* 582 1975
6 Kjösen B Bassoe H H & Myking O The glucose oxidation in isolated leukocytes from female patients suffering from overweight or anorexia nervosa *Scand J Clin Lab Invest 35* 447 1975
7 Laurell C B Electroimmunoassay *Scand J Clin Lab Invest 29* Suppl 124 21 1972
8 McFarlane H Acute phase proteins in malnutrition *In* R M Suskind (ed) *Malnutrition and the Immune Response* Raven Press New York 1977 p 403
9 Palmblad J Fasting (acute energy deprivation) in man effect on polymorphonuclear granulocyte functions plasma iron and serum transferrin *Scand J Hematol 17* 217 1976
10 Palmblad J Fohlin L & Lundstrom M Anorexia nervosa and polymorphonuclear (PMN) granulocyte reactions *Scand J Hematol 19* 334 1977
11 Palmblad J Cantell K Holm G Norberg R Strander H & Sundblad L Acute energy deprivation in man effect on serum immunoglobulins antibody response complement factors 3 & 4 acute phase reactants and interferon producing capacity of blood lymphocytes *Clin Exp Immunol 30* 50 1977
12 Seth V & Chandra R K Opsonic activity phagocytosis and bactericidal capacity of polymorphs in undernutrition *Arch Dis Child 47* 282 1972
13 Sirisinha S Suskind R M Edelman R Kulapongs P & Olson R E The complement system in protein calorie malnutrition—a review *In* R M Suskind (ed) *Malnutrition and the Immune Response* Raven Press New York 1977 p 309

SHORT COMMUNICATION

HEREDITARY TYROSINAEMIA AND DIABETES MELLITUS

The main features in hereditary tyrosinaemia are liver damage leading to cirrhosis and renal tubular defects with hypophosphataemic rickets (4) Pain attacks in the abdomen and legs similar to those seen in acute intermittent porphyria and hypertensive crisis have been reported in this disorder (4) We report here a boy with similar symptoms and signs but who also had diabetes mellitus

Patient

Boy first child to healthy unrelated parents no heredity for diabetes mellitus Birth weight 3180 g After weaning at 3 months he vomited and had a peculiar odour Admitted at 6 months because of fever and abdominal pain He had hepato-splenomegaly and foeter hepaticus Reduced liver produced coagulation factors (e g P & P 5%) hypophosphataemia (0 8 mmol/l) glucosuria generalized aminoaciduria with high excretion of methionine tyrosine and phenolic acids increased urinary excretion of δ aminolevulic acid porphobilinogen in urine negative P tyrosine and P methionine increased (Table 1) Phenylalanine tolerance test (100 mg/kg body weight) gave a maximum value of P tyrosine of 883 μmol/l at 5 h and a slow decrease to 552 μmol/l at 74 h Correspondingly an increased urinary excretion of phenolic acids was observed Fructose tolerance test (50 mg/kg) was normal P-ceruloplasmin and P-orosomucoid levels were remarkably low and P albumin moderately decreased (Table 1) Skeletal X ray showed no signs of rickets He was treated with a tyrosine and phenylalanine restricted diet supplemented with iron as orbic acid vitamin D and other vitamins Marked clinical improvement with increasing levels of coagulation factors (e g P & P 4%) normophosphataemia and reduced levels of P tyrosine but P-ceruloplasmin and P-orosomucoid were still low (Table 1) From 15 months hypophosphataemia increasing urinary volume no glucosuria At 19 months increasing thirst and large urine volume hyperglycaemia (45 mmol/l) glucosuria but no keto acidosis P-insulin 3 μE/ml The same low level of P-ceruloplasmin and P-orosomucoid otherwise normal plasma protein electrophoresis (Table 1) Insulin-dependent diabetes mellitus was diagnosed requiring 12 U insulin per day The diet was modified to satisfy as far as possible the demands of the two diseases During the next year he was in a remarkably good condition and had a normal motoric and mental development his weight was 1 5 kg and height 87 cm at age 2 5 years At 2½ years he had his first hypokalaemic crisis (9 mmol/l) with acidosis At 3 years pain in abdomen and legs ketoacidosis and hypokalaemia blood pressure 130/100 mmHg Urinary volume 3–4 l/day Insulin requirement between 10 and 70 U per day P-ceruloplasmin and P orosomucoid decreased (Table 1) During the next three years he had 12 similar attacks with pains predominantly in the legs hypokalaemia (lowest value 1 4 mmol/l) hypophosphataemia renal acidosis and urinary volume up to 7 l/day At four years radiological signs of rickets and also some pathological fractures Increasing muscle wastage and inability to walk Supplementary large doses of potassium vitamin D phosphate and bicarbonate were tried orally At 6 years he also had a hypertensive crisis blood pressure 160/1 0 mmHg and cerebral symptoms Increased levels of P aldosterone P renin and increased urinary excretion of aldosterone but not of catecholamines At 6½ years he had a hypertensive crisis 230/150 mmHg with encephalopathy and increased urinary excretion of aldosterone and catecholamines he died in a picture of cardiac failure Autopsy was made 48 h after death Advanced autolytic changes made a detailed microscopical investigation impossible The main gross lesions were a nodular cirrhosis of the liver a hypertrophy of the left side of the heart and slight signs of cardiac failure with a moderate pulmonary edema and marked congestion of the liver and the spleen As far as could be seen the islets of Langerhans and the suprarenal glands were normal Some crystalline granules were found in the renal tubular epithelium (Autopsy performed by Dr I Glifberg Department of Pathology Malmo General Hospital Malmo)

This patient fulfils the diagnostic criteria for hereditary tyrosinaemia of the acute type (7) He also had hypertensive crisis intermittent pains and increased levels of P aldosterone and P renin The mechanism for these abnormalities will be discussed more fully elsewhere (to be published)

The isolated decrease of P-ceruloplasmin and P-orosomucoid has not been reported earlier in this disorder The cause of this abnormality is obscure

Diabetes mellitus has not been reported before in hereditary tyrosinaemia Several of the biochemical abnormalities in hereditary tyrosinaemia such as hypokalaemia acidosis hypophosphataemia and hypermethionae

Table 1 Amino acid concentration in plasma (µmol/l) and ceruloplasmin orosomucoid and albumin in plasma (g/l) at various ages

	Reference values (range)		Patient				
	9 m–2 y	4 y–6 y	6 m	1 y	1 y 7 m	3 y	6 y
Tyrosine	11–122	25–58	311	166	65	276	261
Phenylalanine	23–69	38–67	86	36	63	66	46
Methionine	3–29	11–26	496			34	
Isoleucine	26–94	40–63	19		22	85	
Leucine	45–155	75–115	33		31	151	
Arginine	11–65	40–102	45		117	171	
Ceruloplasmin	0.22–0.48		0.06	0.06	0.06	0.15	
Orosomucoid	0.55–1.05		0.16	0.16	0.13	0.3[illegible]	
Albumin	40–52		31	40	39	43	

mia can theoretically contribute to the pathogenesis of diabetes mellitus

A hereditary tyrosinaemia like syndrome i e elevated blood tyrosine and methionine levels and pancreatic islet hyperplasia and degeneration was produced by feeding guinea pigs methionine (6) In Chinese hamsters imbalance in the content of sulfhydryl (SH) containing amino acids caused by excess amounts of methionine (administered intraperitoneally) induced decreased granulation and necrosis of the pancreatic β cells (1) and hyperglycaemia and glucosuria (2) Hypermethioninaemia is also found in patients with homocystinuria Hyperinsulism and also prediabetic oral and intravenous glucose tolerance tests have been observed in such patients (2 3) Furthermore it is well known that several amino acids (mainly arginine) stimulate the secretion of insulin In hereditary tyrosinaemia the pancreatic islets are hyperplastic and hypoglycaemia has been reported (4) Thus it is possible that in hereditary tyrosinaemia after an initial stimulation of the β-cells an exhaustion occurs with hypoinsulism and ensuing diabetes mellitus

The boy had also hypophosphataemia This leads to a state of endogenous insulin resistance (5) This mechanism might have contributed to the exhaustion of the pancreatic β-cells

T Lindberg K O Nilsson J O Jeppsson

Departments of Paediatrics and Clinical Chemistry
Malmo General Hospital
S 21401 Malmo
Sweden

REFERENCES

1 Boquist L The effect of excess methionine on the pancreas A light and electron microscopic study in the Chinese hamster with particular reference to degenerative changes *Lab Invest 21* 96 1969

2 Falkmer S Boquist L Eriksson L Helgesson K Holmgren G & Noren E *Is hypermethioninaemia diabetogenic?* A clinical study in patients with homocystinuria supplemented by experimental investigations in the Chinese hamster *Acta Endocrinol 82* Suppl 203 18 1976

3 Holmgren G Falkmer S & Hambræus L Plasma insulin content and glucose tolerance in homocystinuria *Upsala J Med Sci 78* 215 1973

4 La Du B N & Gjessing L R Tyrosinosis and tyrosinaemia *In* J B Stanbury J B Wyngaarden & D S Fredrickson (eds) *The metabolic basis of inherited disease* 4th ed McGraw Hill Book Co New York 1978 p 256

5 Marshall W P Banasiak M F & Kalkhoff R K Effects of phosphate deprivation on carbohydrate metabolism *Horm Metab Res 10* 369 1978

6 Perry T L Hardwick D F Hansen S Pohlmann L & Warrington P D Methionine induction of experimental tyrosinaemia *J Mental Def Res 11* 246 1967

CASE REPORT

ELECTROLYTE ABNORMALITIES IN LYMPHOSARCOMA AFTER CHEMOTHERAPY

F TZORTZATOU C DACOU VOUTETAKIS S HAIDAS
F PAPADELLIS and TH THOMAIDIS

*From the first Department of Paediatrics of Athens University
St Sophie s Children s Hospital Athens Greece*

ABSTRACT Tzortzatou F Dacou Voutetakis C Haidas S Papadellis F and Thomaidis Th (First Department of Paediatrics of Athens University St Sophie s Children s Hospital Athens Greece) Electrolyte abnormalities in lymphosarcoma after chemotherapy Acta Paediatr Scand 68 621 1979 —A 12 year-old female with lymphosarcoma responding to treatment including vincristine and cyclophosphamide developed clinical and laboratory findings compatible with the syndrome of inappropriate secretion of antidiuretic hormone Some additional findings were observed i e uremia hypopotassemia and alkalosis that have not so far been recorded in that syndrome All abnormalities were corrected upon water restriction A similar episode occurred after a 2nd drug course It too was corrected upon water restriction The patient was clinically free from her malignancy in both episodes It is suggested that our child had probably an expanded form of the syndrome of inappropriate secretion of antidiuretic hormone

KEY WORDS Inappropriate secretion of antidiuretic hormone vincristine cyclophosphamide

Hyponatremia and water intoxication have been described in patients with various malignancies and have been attributed to oversecretion of antidiuretic hormone (ADH) or ADH like substances by tumour cells (4) Vincristine and/or cyclophosphamide toxicity have also been incriminated as causes of the inappropriate antidiuretic hormone secretion syndrome (SIADH) (3 7 8)

The present report concerns a 12 year-old female with a lymphosarcoma in therapeutic remission who presented the syndrome of inappropriate secretion of antidiuretic hormone Attention is drawn to a few laboratory features not so far recorded

CASE REPORT

A 1 year-old female presented with lymphosarcoma of the left parotid gland proven by biopsy which regre sed completely after r diotherapy The parents refused chemotherapy at that time Three months later the patient was first admitted to our service because of a mass arising from the liver pallor anorexia and weight loss Her admission body weight was ?9 kg Investigations showed the following Hemoglobin ?0 g/l leucocytes 8 6 10^9/l neutrophils 94% lymphocytes 5% eosinophils 1% Platelet count was normal as were bone marrow smear chest X ray and intravenous pyelography Urine analysis revealed no pathologic changes specific gravity was 1 0 0 Total serum bilirubin was 35 9 μmol/l unconjugated bilirubin was 22 2 μmol/l SGPT was 34 units and SGOT 100 units Blood urea nitrogen was 6 4 mmol/l She was considered to have a metastasis in the liver She was given a blood transfu ion prednisone mg per kg of body weight for 5 days and the following chemotherapy in one i v dose cyclophosphamide 600 mg per m of body surface i e 750 mg vincristine 1 ? mg per m i e 1 4 mg and adriamycin 40 mg per m² i e 50 mg Two weeks later chemotherapy was repeated once more The liver mass had disappeared completely

Twelve days after the 2nd dose of chemotherapy the patient was anorectic and presented an acute episode of vomiting dyspnea and cyanosis The blood pressure was 60/40 mmHg the pulse was weak and the pul e rate was 80 per min She was started on i v fluid Electrocardiogram showed S–T changes suggestive of myocardial ischaemia and chest X-ray was normal Two hours later

Table 1 *Amino acid concentration in plasma (µmol/l) and ceruloplasmin orosomucoid and albumin in plasma (g/l) at various ages*

	Reference values (range)		Patient				
	9 m–2 y	4 y–6 y	6 m	1 y	1 y 7 m	3 y	6 y
Tyrosine	11–122	25– 58	311	166	65	276	61
Phenylalanine	23– 69	38– 67	86	36	63	66	46
Methionine	3– 29	11– 26	496			34	
Isoleucine	26– 94	40– 63	19		22	85	
Leucine	45–155	75–115	33		31	151	
Arginine	11– 65	40–102	45		117	171	
Ceruloplasmin	0 22–0 48		0 06	0 06	0 06	0 15	
Orosomucoid	0 55–1 05		0 16	0 16	0 13	0 32	
Albumin	40–52		31	40	39	43	

min can theoretically contribute to the pathogenesis of diabetes mellitus

A hereditary tyrosinaemia like syndrome i e elevated blood tyrosine and methionine levels and pancreatic islet hyperplasia and degeneration was produced by feeding guinea pigs methionine (6) In Chinese hamsters imbalance in the content of sulfhydryl (SH) containing amino acids caused by excess amounts of methionine (administered intraperitoneally) induced decreased granulation and necrosis of the pancreatic β cells (1) and hyperglycaemia and glucosuria (2) Hypermethioninaemia is also found in patients with homocystinuria Hyperinsulism and also prediabetic oral and intravenous glucose tolerance tests have been observed in such patients (2, 3) Furthermore it is well known that several amino acids (mainly arginine) stimulate the secretion of insulin In hereditary tyrosinaemia the pancreatic islets are hyperplastic and hypoglycaemia has been reported (4) Thus it is possible that in hereditary tyrosinaemia after an initial stimulation of the β cells an exhaustion occurs with hypoinsulism and ensuing diabetes mellitus

The boy had also hypophosphataemia This leads to a state of endogenous insulin resistance (5) This mechanism might have contributed to the exhaustion of the pancreatic β-cells

T Lindberg K O Nilsson J O Jeppsson

Departments of Paediatrics and Clinical Chemistry
Malmo General Hospital
S 214 01 Malmo
Sweden

REFERENCES

1 Boquist L The effect of excess methionine on the pancreas A light and electron microscopic study in the Chinese hamster with particular reference to degenerative changes *Lab Invest 21* 96 1969
2 Falkmer S Boquist L Eriksson L Helgesson K Holmgren G & Norén E Is hypermethioninaemia diabetogenic? A clinical study in patients with homocystinuria supplemented by experimental investigations in the Chinese hamster *Acta Endocrinol 82 Suppl 203* 18 1976
3 Holmgren G Falkmer S & Hambræus L Plasma insulin content and glucose tolerance in homocystinuria *Upsala J Med Sci 78* 215 1973
4 La Du B N & Gjessing L R Tyrosinosis and tyrosinaemia *In* J B Stanbury J B Wyngaarden & D S Fredrickson (eds) *The metabolic basis of inherited disease* 4th ed McGraw Hill Book Co New York 1978 p 256
5 Marshall W P Banasiak M F & Kalkhoff R K Effects of phosphate deprivation on carbohydrate metabolism *Horm Metab Res 10* 369 1978
6 Perry T L Hardwick D F Hansen S Pohlmann L & Warrington P D Methionine induction of experimental tyrosinaemia *J Mental Def Res 11* 246 1967

Acta Pædiatr Scand 68 621–623 1979

CASE REPORT

ELECTROLYTE ABNORMALITIES IN LYMPHOSARCOMA AFTER CHEMOTHERAPY

F TZORTZATOU C DACOU VOUTETAKIS S HAIDAS
F PAPADELLIS and TH THOMAIDIS

*From the first Department of Pediatrics of Athens University
St Sophie s Children s Hospital Athens Greece*

ABSTRACT Tzortzatou F Dacou Voutetakis C Haidas S Papadellis F and Thomaidi Th (First Department of Paediatrics of Athens University St Sophie s Children s Hospital Athens Greece) Electrolyte abnormalities in lymphosarcoma after chemotherapy Acta Paediatr Scand 68 621 1979 —A 12 year-old female with lymphosarcoma responding to treatment including vincristine and cyclophosphamide developed clinical and laboratory findings compatible with the syndrome of inappropriate secretion of antidiuretic hormone Some additional findings were observed i e uremia hypopotassemia and alkalosis that have not so far been recorded in that syndrome All abnormalities were corrected upon water restriction A similar episode occurred after a 2nd drug course It too was corrected upon water restriction The patient was clinically free from her malignancy in both episodes It is suggested that our child had probably an expanded form of the syndrome of inappropriate secretion of antidiuretic hormone

KEY WORDS Inappropriate secretion of antidiuretic hormone vincristine cyclophosphamide

Hyponatremia and water intoxication have been described in patients with various malignancies and have been attributed to oversecretion of antidiuretic hormone (ADH) or ADH like substances by tumour cells (4) Vincristine and/or cyclophosphamide toxicity have also been incriminated as causes of the inappropriate antidiuretic hormone secretion syndrome (SIADH) (3 7 8)

The present report concerns a 12 year-old female with a lymphosarcoma in therapeutic remission who presented the syndrome of inappropriate secretion of antidiuretic hormone Attention is drawn to a few laboratory features not so far recorded

CASE REPORT

A 1 year-old female presented with lymphosarcoma of the left parotid gland proven by biopsy which regressed completely after radiotherapy The parents refused chemotherapy at that time Three months later the patient was first admitted to our service because of a mass arising from the liver pallor anorexia and weight loss Her admission body weight was 29 kg Investigations showed the following Hemoglobin 70 g/l leucocytes 8 6 10⁹/l neutrophils 94 % lymphocytes 5 % eosinophils 1 % Platelet count was normal as were bone marrow smear chest X ray and intravenous pyelography Urine analysis revealed no pathologic changes specific gravity was 1 0 0 Total serum bilirubin was 35 9 μmol/l unconjugated bilirubin was 27 2 μmol/l SGPT was 34 units and SGOT 100 units Blood urea nitrogen was 6 4 mmol/l She was considered to have a metastasis in the liver She was given a blood transfusion prednisone 2 mg per kg of body weight for 5 days and the following chemotherapy in one i v dose cyclophosphamide 600 mg per m² of body surface i e 750 mg vincristine 1 2 mg per m² i e 1 4 mg and adriamycin 40 mg per m² i e 50 mg Two weeks later chemotherapy was repeated once more The liver mass had disappeared completely

Twelve days after the 2nd dose of chemotherapy the patient was anorectic and presented an acute episode of vomiting dyspnea and cyanosis The blood pressure was 60/40 mmHg the pulse was weak and the pulse rate was 80 per min She was started on i v fluids Electrocardiogram showed S–T changes suggestive of myocardial ischaemia and chest X ray was normal Two hours later

Table 1 *Amino acid concentration in plasma (µmol/l) and ceruloplasmin orosomucoid and albumin in plasma (g/l) at various ages*

	Reference values (range)		Patient				
	9 m–2 y	4 y–6 y	6 m	1 y	1 y 7 m	3 y	6 y
Tyrosine	11–122	25–58	311	166	65	276	261
Phenylalanine	23–69	38–67	86	36	63	66	46
Methionine	3–29	11–26	496			34	
Isoleucine	26–94	40–63	19		22	85	
Leucine	45–155	75–115	33		31	151	
Arginine	11–65	40–102	45		117	121	
Ceruloplasmin	0.22–0.48		0.06	0.06	0.06	0.15	
Orosomucoid	0.55–1.05		0.16	0.16	0.13	0.32	
Albumin	40–52		31	40	39	43	

mia can theoretically contribute to the pathogenesis of diabetes mellitus

A hereditary tyrosinaemia like syndrome i e elevated blood tyrosine and methionine levels and pancreatic islet hyperplasia and degeneration was produced by feeding guinea pigs methionine (6) In Chinese hamsters imbalance in the content of sulfhydryl (SH) containing amino acids caused by excess amounts of methionine (administered intraperitoneally) induced decreased granulation and necrosis of the pancreatic β cells (1) and hyperglycaemia and glucosuria (2) Hypermethioninaemia is also found in patients with homocystinuria Hyperinsulism and also prediabetic oral and intravenous glucose tolerance tests have been observed in such patients (2 3) Furthermore it is well known that several amino acids (mainly arginine) stimulate the secretion of insulin In hereditary tyrosinaemia the pancreatic islets are hyperplastic and hypoglycaemia has been reported (4) Thus it is possible that in hereditary tyrosinaemia after an initial stimulation of the β cells an exhaustion occurs with hypoinsulism and ensuing diabetes mellitus

The boy had also hypophosphataemia This leads to a state of endogenous insulin resistance (5) This mechanism might have contributed to the exhaustion of the pancreatic β-cells

T Lindberg K O Nilsson J O Jeppsson

Departments of Paediatrics and Clinical Chemistry
Malmo General Hospital
S 214 01 Malmo
Sweden

REFERENCES

1 Boquist L The effect of excess methionine on the pancreas A light and electron microscopic study in the Chinese hamster with particular reference to degenerative changes *Lab Invest* 21 96 1969
2 Falkmer S Boquist L Eriksson L Helgesson K Holmgren G & Norén E Is hypermethioninaemia diabetogenic? A clinical study in patients with homocystinuria supplemented by experimental investigations in the Chinese hamster *Acta Endocrinol* 82 Suppl 203 18 1976
3 Holmgren G Falkmer S & Hambræus L Plasma insulin content and glucose tolerance in homocystinuria *Upsala J Med Sci* 78 215 1973
4 La Du B N & Gjessing L R Tyrosinosis and tyrosinaemia *In* J B Stanbury J B Wyngaarden & D S Fredrickson (eds) *The metabolic basis of inherited disease* 4th ed McGraw Hill Book Co New York 1978 p 256
5 Marshall W P Banasiak M F & Kalkhoff R K Effects of phosphate deprivation on carbohydrate metabolism *Horm Metab Res* 10 369 1978
6 Perry T L Hardwick D F Hansen S Pohlmann L & Warrington P D Methionine induction of experimental tyrosinaemia *J Mental Def Res* 11 246 1967

CASE REPORT

ELECTROLYTE ABNORMALITIES IN LYMPHOSARCOMA AFTER CHEMOTHERAPY

F TZORTZATOU C DACOU VOUTETAKIS S HAIDAS
F PAPADELLIS and TH THOMAIDIS

From the first Department of Paediatrics of Athens University St Sophie s Children s Hospital Athens Greece

ABSTRACT Tzortzatou F Dacou Voutetakis C Haidas S Papadellis F and Thomaidis Th (First Department of Paediatrics of Athens University St Sophie s Children s Hospital Athens Greece) Electrolyte abnormalities in lymphosarcoma after chemotherapy Acta Paediatr Scand 68 621 1979 —A 12-year-old female with lymphosarcoma responding to treatment including vincristine and cyclophosphamide developed clinical and laboratory findings compatible with the syndrome of inappropriate secretion of antidiuretic hormone Some additional findings were observed i e uremia hypopotassemia and alkalosis that have not so far been recorded in that syndrome All abnormalities were corrected upon water restriction A similar episode occurred after a 2nd drug course It too was corrected upon water restriction The patient was clinically free from her malignancy in both episodes It is suggested that our child had probably an expanded form of the syndrome of inappropriate secretion of antidiuretic hormone

KEY WORDS Inappropriate secretion of antidiuretic hormone vincristine cyclophosphamide

Hyponatremia and water intoxication have been described in patients with various malignancies and have been attributed to oversecretion of antidiuretic hormone (ADH) or ADH like substances by tumour cells (4) Vincristine and/or cyclophosphamide toxicity have also been incriminated as causes of the inappropriate antidiuretic hormone secretion syndrome (SIADH) (3 7 8)

The present report concerns a 12 year-old female with a lymphosarcoma in therapeutic remission who presented the syndrome of inappropriate secretion of antidiuretic hormone Attention is drawn to a few laboratory features not so far recorded

CASE REPORT

A 1 year-old female presented with lymphosarc ma of the left parotid gland proven by biopsy which regressed completely after radiotherapy The parents refused chemotherapy at that time Three months later the patient was first admitted to our service because of a mass arising from the liver pallor anorexia and weight loss Her admission body weight was 9 kg Investigations showed the following Hemoglobin 70 g/l leucocytes 8 6 10^9/l neutrophils 94% lymphocytes 5% eosinophils 1% Platelet count was normal as were bone marrow smear chest X ray and intravenous pyelography Urine analysis revealed no pathologic changes specific gravity was 1 0?0 Total serum bilirubin was 35 9 μmol/l unconjugated bilirubin was 2 ? μmol/l SGPT was 34 units and SGOT 100 units Blood urea nitrogen was 6 4 mmol/l She was considered to have a metastasis in the liver She was given a blood transfusion prednisone ? mg per kg of body weight for 5 days and the following chemotherapy in one i v dose cyclophosphamide 600 mg per m of body surface i e 7?0 mg vincristine 1 ? mg per m i e 1 4 mg and adriamycin 40 mg per m² i e ?0 mg Two weeks later chemotherapy was repeated once more The liver mass had disappeared completely

Twelve days after the nd dose of chemotherapy the patient was anorectic and presented an acute episode of vomiting dyspnea and cyanosis The blood pressure was 60/40 mmHg the pulse was weak and the pulse rate was 80 per min She was started on i v fluids Electrocardiogram showed S–T changes suggestive of myocardial ischaemia and chest X-ray was normal Two hours later

Table I *Amino acid concentration in plasma (µmol/l) and ceruloplasmin orosomucoid and albumin in plasma (g/l) at various ages*

	Reference values (range)		Patient				
	9 m–2 y	4 y–6 y	6 m	1 y	1 y 7 m	3 y	6 y
Tyrosine	11–122	25–58	311	166	65	276	261
Phenylalanine	23–69	38–67	86	36	63	66	46
Methionine	3–29	11–26	496			34	
Isoleucine	26–94	40–63	19		22	85	
Leucine	45–155	75–115	33		31	151	
Arginine	11–65	40–102	45		117	121	
Ceruloplasmin	0.22–0.48		0.06	0.06	0.06	0.15	
Orosomucoid	0.55–1.05		0.16	0.16	0.13	0.32	
Albumin	40–52		31	40	39	43	

mia can theoretically contribute to the pathogenesis of diabetes mellitus

A hereditary tyrosinaemia like syndrome i e elevated blood tyrosine and methionine levels and pancreatic islet hyperplasia and degeneration was produced by feeding guinea pigs methionine (6) In Chinese hamsters imbalance in the content of sulfhydryl (SH) containing amino acids caused by excess amounts of methionine (administered intraperitoneally) induced decreased granulation and necrosis of the pancreatic β-cells (1) and hyperglycaemia and glucosuria (2) Hypermethioninaemia is also found in patients with homocystinuria Hyperinsulism and also prediabetic oral and intravenous glucose tolerance tests have been observed in such patients (2 3) Furthermore it is well known that several amino acids (mainly arginine) stimulate the secretion of insulin In hereditary tyrosinaemia the pancreatic islets are hyperplastic and hypoglycaemia has been reported (4) Thus it is possible that in hereditary tyrosinaemia after an initial stimulation of the β cells an exhaustion occurs with hypoinsulism and ensuing diabetes mellitus

The boy had also hypophosphataemia This leads to a state of endogenous insulin resistance (5) This mechanism might have contributed to the exhaustion of the pancreatic β-cells

T Lindberg   K O Nilsson   J O Jeppsson

Departments of Paediatrics and Clinical Chemistry
Malmo General Hospital
S 214 01 Malmo
Sweden

REFERENCES

1 Boquist L The effect of excess methionine on the pancreas A light and electron microscopic study in the Chinese hamster with particular reference to degenerative changes *Lab Invest 21* 96 1969
2 Falkmer S Boquist L Eriksson L Helgesson K Holmgren G & Noren E Is hypermethioninaemia diabetogenic? A clinical study in patients with homocystinuria supplemented by experimental investigations in the Chinese hamster *Acta Endocrinol 82* Suppl *203* 18 1976
3 Holmgren G Falkmer S & Hambræus L Plasma insulin content and glucose tolerance in homocystinuria *Upsala J Med Sci 78* 215 1973
4 La Du B N & Gjessing L R Tyrosinosis and tyrosinaemia *In* J B Stanbury J B Wyngaarden & D S Fredrickson (eds) *The metabolic basis of inherited disease* 4th ed McGraw Hill Book Co New York 1978 p 256
5 Marshall W P Banasiak M F & Kalkhoff R K Effects of phosphate deprivation on carbohydrate metabolism *Horm Metab Res 10* 369 1978
6 Perry T L Hardwick D F Hansen S Pohlmann L & Warrington P D Methionine induction of experimental tyrosinaemia *J Mental Def Res 11* 246 1967

CASE REPORT

ELECTROLYTE ABNORMALITIES IN LYMPHOSARCOMA AFTER CHEMOTHERAPY

F TZORTZATOU C DACOU VOUTETAKIS S HAIDAS
F PAPADELLIS and TH THOMAIDIS

From the first Department of Paediatrics of Athens University St Sophie s Child en s Hospital Athens Greece

ABSTRACT Tzortzatou F Dacou Voutetakis C Haidas S Papadellis F and Thomaidis Th (First Department of Paediatrics of Athens University St Sophie s Children s Hospital Athens Greece) Electrolyte abnormalities in lymphosarcoma after chemotherapy Acta Paediatr Scand 68 621 1979 —A 12-year-old female with lymphosarcoma responding to treatment including vincristine and cyclophosphamide developed clinical and laboratory findings compatible with the syndrome of inappropriate secretion of antidiuretic hormone Some additional findings were observed i e uremia hypopotassemia and alkalosis that have not so far been recorded in that syndrome All abnormalities were corrected upon water restriction A similar episode occurred after a 2nd drug course It too was corrected upon water restriction The patient was clinically free from her malignancy in both episodes It is suggested that our child had probably an expanded form of the syndrome of inappropriate secretion of antidiuretic hormone

KEY WORDS Inappropriate secretion of antidiuretic hormone vincristine cyclophosphamide

Hyponatremia and water intoxication have been described in patients with various malignancies and have been attributed to oversecretion of antidiuretic hormone (ADH) or ADH like substances by tumour cells (4) Vincristine and/or cyclophosphamide toxicity have also been incriminated as causes of the inappropriate antidiuretic hormone secretion syndrome (SIADH) (3 7 8)

The present report concerns a 12 year-old female with a lymphosarcoma in therapeutic remission who presented the syndrome of inappropriate secretion of antidiuretic hormone Attention is drawn to a few laboratory features not so far recorded

CASE REPORT

A 1 year-old female presented with lymphosarcoma of the left parotid gland proven by biopsy which regressed completely after radiotherapy The parents refused chemotherapy at that time Three months later the patient was first admitted to our service because of a mass arising from the liver pallor anorexia and weight loss H r admission body weight was 9 kg Investigations showed the following Hemoglobin 70 g/l leucocytes 8 6 10⁹/l neutrophils 94% lymphocytes 5% eosinophils 1% Platelet count was normal as were bone marrow smear chest X ray and intravenous pyelography Urine analysis revealed no pathologic changes specific gravity was 1 0?0 Total serum bilirubin was 35 9 μmol/l unconjugated bilirubin was 2 ? μmol/l SGPT was 34 units and SGOT 100 units Blood urea nitrogen was 6 4 mmol/l She was considered to have a metastasis in the liver She was given a blood transfusion prednisone 2 mg per kg of body weight for 5 days and the following chemotherapy in one i v dose cyclophosphamide 600 mg per m² of body surface i e 750 mg vincristine 1 2 mg per m i e 1 4 mg and adriamycin 40 mg per m² i e 50 mg Two weeks later chemotherapy was repeated once more The liver mass had disappeared completely

Twelve days after the nd dose of chemotherapy the patient was anorectic and presented an acute episode of vomiting dyspnea and cyanosis The blood pressure was 60/40 mmHg the pulse was weak and the pulse rate was 80 per min She was started on i v fluids Electrocardiogram showed S-T changes suggestive of myocardial is haemia and chest X ray was normal Two hours later

the blood pressure was 80/60 mmHg. The episode was initially attributed to the toxic effect of adriamycin on the myocardium. A few hours later the blood pressure was restored and cyanosis had disappeared but all other symptoms persisted. At that stage the following laboratory data were obtained: serum sodium (Na) was 127 mmol/l, potassium (K) 2.5 mmol/l and chloride (Cl) 60 mmol/l, carbon dioxide content (CO_2) 62.4 mmol/l, blood pH 7.55 and urine pH 8. Blood urea nitrogen was 35 mmol/l, creatinine ranged from 80 to 96 μmol/l, blood glucose was *5.7 mmol/l*, serum calcium 5 mmol/l and phosphate 1.3 mmol/l. She was immediately started on NaCl 0.9% i.v. 3000 cc per 24 hours and 105 mEq/l potassium per 24 hours which was given for 3 days without any biochemical improvement. At that stage the diagnosis of inappropriate ADH secretion was entertained and strongly considered when the serum osmolality was found to be 268 mosm/kg and that of urine 811 mosm/kg. The urine specific gravity was 1.021 and the rest of the urine analysis was normal. Antidiuretic hormone could not be measured. Subsequently the fluids were restricted *to a total of 600 ml per 24 hours*. Two days later all electrolytes, pH and blood urea were restored to normal and the patient was discharged in good condition. One month later she was readmitted to continue her treatment. She received the same chemotherapy except for adriamycin as E.C.G. changes persisted. After 17 days she developed again the same clinical and laboratory abnormalities (Na 128 mmol/l, Cl 66 mmol/l, K 1.6 mmol/l, CO_2 54 mmol/l and blood urea nitrogen 27.5 mmol/l) which improved with fluid restriction.

DISCUSSION

The accepted clinical and laboratory criteria for the diagnosis of the syndrome of inappropriate antidiuretic hormone secretion (SIADH) are the following: (*a*) hyponatremia and hypo-osmolality of serum and extracellular fluid, (*b*) increased urinary sodium and hyper osmolality in relation to that of serum, (*c*) low serum chloride, (*d*) no clinical evidence of hypovolemia, dehydration and edema, (*e*) no renal nor adrenal disease and (*f*) *improvement* of the above upon water restriction (4, 6, 7).

Our child probably represents a case of SIADH since she satisfies all of the above criteria. Her severely depleted electrolytes *were* indeed not restored by intravenous administration of 0.9% NaCl solution and KCl; they were corrected by simple restriction *of fluids*. On the other hand, the absence of albuminuria, glucosuria, hyposthenuria and increased serum creatinine exclude renal disease; hypopotassemia without polyuria would also tend to exclude adrenal disease.

Our child, however, had in addition uremia, low serum potassium (2.5 mmol/l) and metabolic alkalosis (CO_2 62.4 mmol/l and blood pH 7.55). Whether these represent an expansion of the syndrome itself or some other associated abnormalities of obscure nature cannot be stated at present. Uremia was not renal since it was transient and not accompanied with other renal findings. It was observed only during both episodes. Low serum potassium has been observed in leukemia and other early or advanced lymphocellular malignancies (4) as well as after prolonged antibiotic administration (5). Our child did not receive antibiotics and her malignancy was in definite remission at the time. Alkalosis has been said to occur secondary to low serum potassium as a compensatory mechanism or as others believe hypopotassemia is due to the alkalosis itself. What is however impressive is the disappearance of uremia, hypopotassemia and alkalosis upon water restriction and the subsequent remission of the syndrome. The clinical and laboratory features of this syndrome not only disappeared after water restriction but reappeared when the same chemotherapy was given again a few weeks later to be restored once more after water restriction. During the 2nd episode uremia, hypopotassemia and alkalosis were also present. E.C.G. changes were attributed to adriamycin and not to hypopotassemia since the latter was corrected whereas the E.C.G. changes persisted.

As far as causation is concerned a multitude of factors have been incriminated. SIADH may be due to the tumour itself (4) or to some other pathological state. It is believed that tumour cells synthesize and secrete a substance that simulates antidiuretic hormone in chemical composition and pharmacologic action but is not detectable by the specific radioimmunoassay. On the other hand, both vincristine and cyclophosphamide have the same effect on the hypothalamus and/or the hypothalamic-pituitary pathway causing ab-

normal secretion of ADH which continues to act paradoxically since hyponatremia would be expected to suppress ADH secretion Cases have been recorded in which ADH was found at high levels by radioimmunoassay (7) Unfortunately ADH was not measured in our patient In our child the drugs vincristine and/or cyclophosphamide must have caused the electrolyte disturbance since our patient was at the time in remission from her malignancy and the episode recurred when the drugs were given again

As far as management is concerned some authors have in addition given a diuretic (2) e g furosemide (Lasix) and others have resorted to lithium therapy with encouraging results (1) In our child no maintenance therapy is needed at present

REFERENCES

1 Baker R S Hurley M R & Feldman W Treatment of recurrent syndrome of inappropriate secretion of antidiuretic hormone with lithium *J Pediatr 90* 480 1977

2 Cohen L F Di Sant Agnese P A Taylor A & Gill J R The syndrome of inappropriate antidiuretic hormone secretion as a cause of hyponatremia in cystic fibrosis *J Pediatr 9* 574 1977

3 Fine R N Clarke R R & Shore N A Hyponatremia and vincristine therapy *Am J Dis Child 112* 256 1966

4 O Regan S Carson S Chesney R W & Drummond K N Electrolyte and acid–base disturbances in the management of leukemia *Blood 49* 345 1977

5 Stapleton F B Nelson B Vats T S & Linshaw M A Hypokalemia associated with antibiotic treatment *Am J Dis Child 130* 1104 1976

6 Steele T H Serpick A A & Block J B Antidiuretic response to cyclophosphamide in man *J Pharmacol Exper Ther 185* 245 1973

7 Stuart M J Cuaso C Miller M & Oski F A Syndrome of recurrent increased secretion of antidiuretic hormone following multiple doses of vincristine *Blood 45* 315 1975

8 Weinberg J A Weitzman R E Zakandalin S & Leake R D Inappropriate secretion of antidiuretic hormone in a premature infant *J Pediatr 90* 111 1977

Submitted Oct 7 19 8
Accepted Dec 4 1978

(F T) Department of Paediatrics
St Sophie s Children s Hospital
Athens 608
Greece

the blood pressure was 80/60 mmHg. The episode was initially attributed to the toxic effect of adriamycin on the myocardium. A few hours later the blood pressure was restored and cyanosis had disappeared, but all other symptoms persisted. At that stage the following laboratory data were obtained: serum sodium (Na) was 127 mmol/l, potassium (K) 2.5 mmol/l and chloride (Cl) 60 mmol/l, carbon dioxide content (CO_2) 62.4 mmol/l, blood pH 7.55 and urine pH 8. Blood urea nitrogen was 35 mmol/l, creatinine ranged from 80 to 96 μmol/l, blood glucose was 5.7 mmol/l, serum calcium 5 mmol/l and phosphate 1.3 mmol/l. She was immediately started on NaCl 0.9% i.v. 3000 cc per 24 hours and 105 ml q/l potassium per 24 hours which was given for 3 days without any biochemical improvement. At that stage the diagnosis of inappropriate ADH secretion was entertained and strongly considered when the serum osmolality was found to be 268 mosm./kg and that of urine 811 mosm./kg. The urine specific gravity was 1021 and the rest of the urine analysis was normal. Antidiuretic hormone could not be measured. Subsequently the fluids were restricted to a total of 600 ml per 24 hours. Two days later all electrolytes, pH and blood urea were restored to normal and the patient was discharged in good condition. One month later she was re-admitted to continue her treatment. She received the same chemotherapy except for adriamycin, as E.C.G. changes persisted. After 17 days she developed again the same clinical and laboratory abnormalities (Na 128 mmol/l, Cl 66 mmol/l, K 1.6 mmol/l, CO_2 54 mmol/l and blood urea nitrogen 27.5 mmol/l) which improved with fluid restriction.

DISCUSSION

The accepted clinical and laboratory criteria for the diagnosis of the syndrome of inappropriate antidiuretic hormone secretion (SIADH) are the following: (*a*) hyponatremia and hypo-osmolality of serum and extracellular fluid, (*b*) increased urinary sodium and hyper osmolality in relation to that of serum, (*c*) low serum chloride, (*d*) no clinical evidence of hypovolemia, dehydration and edema, (*e*) no renal nor adrenal disease and (*f*) improvement of the above upon water restriction (4, 6, 7).

Our child probably represents a case of SIADH since she satisfies all of the above criteria. Her severely depleted electrolytes were indeed not restored by intravenous administration of 0.9% NaCl solution and KCl; they were corrected by simple restriction of fluids. On the other hand the absence of albuminuria, glucosuria, hyposthenuria and increased serum creatinine exclude renal disease; hypopotassemia without polyuria would also tend to exclude adrenal disease.

Our child however had in addition uremia, low serum potassium (2.5 mmol/l) and metabolic alkalosis (CO_2 62.4 mmol/l and blood pH 7.55). Whether these represent an expansion of the syndrome itself or some other associated abnormalities of obscure nature cannot be stated at present. Uremia was not renal since it was transient and not accompanied with other renal findings. It was observed only during both episodes. Low serum potassium has been observed in leukemia and other early or advanced lymphocellular malignancies (4) as well as after prolonged antibiotic administration (5). Our child did not receive antibiotics and her malignancy was in definite remission at the time. Alkalosis has been said to occur secondary to low serum potassium as a compensatory mechanism or as others believe, hypopotassemia is due to the alkalosis itself. What is however impressive is the disappearance of uremia, hypopotassemia and alkalosis upon water restriction and the subsequent remission of the syndrome. The clinical and laboratory features of this syndrome not only disappeared after water restriction but reappeared when the same chemotherapy was given again a few weeks later to be restored once more after water restriction. During the 2nd episode uremia, hypopotassemia and alkalosis were also present. E.C.G. changes were attributed to adriamycin and not to hypopotassemia since the latter was corrected whereas the E.C.G. changes persisted.

As far as causation is concerned a multitude of factors have been incriminated. SIADH may be due to the tumour itself (4) or to some other pathological state. It is believed that tumour cells synthesize and secrete a substance that simulates antidiuretic hormone in chemical composition and pharmacologic action but is not detectable by the specific radioimmunoassay. On the other hand both vincristine and cyclophosphamide have the same effect on the hypothalamus and/or the hypothalamic pituitary pathway causing ab-

normal secretion of ADH which continues to act paradoxically since hyponatremia would be expected to suppress ADH secretion Cases have been recorded in which ADH was found at high levels by radioimmunoassay (7) Unfortunately ADH was not measured in our patient In our child the drugs vincristine and/or cyclophosphamide must have caused the electrolyte disturbance since our patient was at the time in remission from her malignancy and the episode recurred when the drugs were given again

As far as management is concerned some authors have in addition given a diuretic (2) e g furosemide (Lasix) and others have resorted to lithium therapy with encouraging results (1) In our child no maintenance therapy is needed at present

REFERENCES

1 Baker R S Hurley M R & Feldman W Treatment of recurrent syndrome of inappropriate secretion of antidiuretic hormone with lithium *J Pediatr 90* 480 1977

2 Cohen L F Di Sant Agnese P A Taylor A & Gill J R The syndrome of inappropriate antidiuretic hormone secretion as a cause of hyponatremia in cystic fibrosis *J Pediatr 9* 574 1977

3 Fine R N Clarke R R & Shore N A Hyponatremia and vincristine therapy *Am J Dis Child 112* 256 1966

4 O Regan S Carson S Chesney R W & Drummond K N Electrolyte and acid-base disturbances in the management of leukemia *Blood 49* 345 1977

5 Stapleton F B Nelson B Vats T S & Linshaw M A Hypokalemia associated with antibiotic treatment *Am J Dis Child 130* 1104 1976

6 Steele T H Serpick A A & Block J B Antidiuretic response to cyclophosphamide in man *J Pharmacol Exper Ther 185* 245 1973

7 Stuart M J Cuaso C Miller M & Oski F A Syndrome of recurrent increased secretion of antidiuretic hormone following multiple doses of vincristine *Blood 45* 315 1975

8 Weinberg J A Weitzman R E Zakandalin S & Leake R D Inappropriate secretion of antidiuretic hormone in a premature infant *J Pediatr 90* 111 1977

Submitted Oct 1978
Accepted Dec 4 1978

(F T) Department of Paediatrics
St Sophie s Children s Hospital
Athens 608
Greece

the blood pressure was 80/60 mmHg. The episode was initially attributed to the toxic effect of adriamycin on the myocardium. A few hours later the blood pressure was restored and cyanosis had disappeared, but all other symptoms persisted. At that stage the following laboratory data were obtained: serum sodium (Na^+) was 127 mmol/l, potassium (K^+) 2.5 mmol/l and chloride (Cl^-) 60 mmol/l, carbon dioxide content (CO_2) 62.4 mmol/l, blood pH 7.55 and urine pH 8. Blood urea nitrogen was 35 mmol/l, creatinine ranged from 80 to 96 μmol/l, blood glucose was 5.7 mmol/l, serum calcium 5 mmol/l and phosphate 1.3 mmol/l. She was immediately started on NaCl 0.9% i.v. 3000 cc per 24 hours and 105 mEq/l potassium per 24 hours which was given for 3 days without any biochemical improvement. At that stage the diagnosis of inappropriate ADH secretion was entertained and strongly considered when the serum osmolality was found to be 268 mosm/kg and that of urine 811 mosm/kg. The urine specific gravity was 1.021 and the rest of the urine analysis was normal. Antidiuretic hormone could not be measured. Subsequently the fluids were restricted to a total of 600 ml per 24 hours. Two days later all electrolytes, pH and blood urea were restored to normal and the patient was discharged in good condition. One month later she was readmitted to continue her treatment. She received the same chemotherapy except for adriamycin as E.C.G. changes persisted. After 17 days she developed again the same clinical and laboratory abnormalities (Na 138 mmol/l, Cl 66 mmol/l, K 1.6 mmol/l, CO_2 54 mmol/l and blood urea nitrogen 27.5 mmol/l) which improved with fluid restriction.

DISCUSSION

The accepted clinical and laboratory criteria for the diagnosis of the syndrome of inappropriate antidiuretic hormone secretion (SIADH) are the following: (*a*) hyponatremia and hypo-osmolality of serum and extracellular fluid, (*b*) increased urinary sodium and hyper osmolality in relation to that of serum, (*c*) low serum chloride, (*d*) no clinical evidence of hypovolemia, dehydration and edema, (*e*) no renal nor adrenal disease and (*f*) improvement of the above upon water restriction (4, 6, 7).

Our child probably represents a case of SIADH, since she satisfies all of the above criteria. Her severely depleted electrolytes were indeed not restored by intravenous administration of 0.9% NaCl solution and KCl; they were corrected by simple restriction of fluids. On the other hand, the absence of albuminuria, glucosuria, hyposthenuria and increased serum creatinine exclude renal disease; hypopotassemia without polyuria would also tend to exclude adrenal disease.

Our child, however, had in addition uremia, low serum potassium (2.5 mmol/l) and metabolic alkalosis (CO_2 62.4 mmol/l and blood pH 7.55). Whether these represent an expansion of the syndrome itself or some other associated abnormalities of obscure nature cannot be stated at present. Uremia was not renal since it was transient and not accompanied with other renal findings. It was observed only during both episodes. Low serum potassium has been observed in leukemia and other early or advanced lymphocellular malignancies (4) as well as after prolonged antibiotic administration (5). Our child did not receive antibiotics and her malignancy was in definite remission at the time. Alkalosis has been said to occur secondary to low serum potassium as a compensatory mechanism or as others believe hypopotassemia is due to the alkalosis itself. What is however impressive is the disappearance of uremia, hypopotassemia and alkalosis upon water restriction and the subsequent remission of the syndrome. The clinical and laboratory features of this syndrome not only disappeared after water restriction but reappeared when the same chemotherapy was given again a few weeks later to be restored once more after water restriction. During the 2nd episode uremia, hypopotassemia and alkalosis were also present. E.C.G. changes were attributed to adriamycin and not to hypopotassemia, since the latter was corrected whereas the E.C.G. changes persisted.

As far as causation is concerned a multitude of factors have been incriminated. SIADH may be due to the tumour itself (4) or to some other pathological state. It is believed that tumour cells synthesize and secrete a substance that simulates antidiuretic hormone in chemical composition and pharmacologic action, but is not detectable by the specific radioimmunoassay. On the other hand, both vincristine and cyclophosphamide have the same effect on the hypothalamus and/or the hypothalamic pituitary pathway, causing ab-

normal secretion of ADH which continues to act paradoxically since hyponatremia would be expected to suppress ADH secretion Cases have been recorded in which ADH was found at high levels by radioimmunoassay (7) Unfortunately ADH was not measured in our patient In our child the drugs vincristine and/ or cyclophosphamide must have caused the electrolyte disturbance since our patient was at the time in remission from her malignancy and the episode recurred when the drugs were given again

As far as management is concerned some authors have in addition given a diuretic (2) e g furosemide (Lasix) and others have re sorted to lithium therapy with encouraging results (I) In our child no maintenance therapy is needed at present

REFERENCES

1 Baker R S Hurley M R & Feldman W Treat ment of recurrent syndrome of inappropriate secretion of antidiuretic hormone with lithium *J Pediatr 90* 480 1977

2 Cohen L F Di Sant Agnese P A Taylor A & Gill J R The syndrome of inappropriate antidiuretic hormone secretion as a cause of hyponatremia in cystic fibrosis *J Pediatr 9* 574 1977

3 Fine R N Clarke R R & Shore N A Hypo natremia and vincristine therapy *Am J Dis Child 11?* ?56 1966

4 O Regan S Carson S Chesney R W & Drum mond K N Electrolyte and acid-base disturbances in the management of leukemia *Blood 49* 345 1977

5 Stapleton F B Nelson B Vats T S & Linshaw M A Hypokalemia associated with antibiotic treat ment *Am J Dis Child 130* 1104 1976

6 Steele T H Serpick A A & Block J B Anti diuretic response to cyclophosphamide in man *J Pharmacol Exper Ther 185* ?45 1973

7 Stuart M J Cuaso C Miller M & Oski F A Syndrome of recurrent increased secretion of antidi uretic hormone following multiple doses of vincristine *Blood 45* 315 1975

8 Weinberg J A Weitzman R E Zakandalin S & Leake R D Inappropriate secretion of antidiuretic hormone in a premature infant *J Pediatr 90* 111 1977

Submitted Oct ? 1978
Accepted Dec 4 1978

(F T) Department of Paediatrics
St Sophie s Children s Hospital
Athens 608
Greece

Acta Pædiatr Scand 68 625–626 1979

CASE REPORT

SPONTANEOUS PERFORATION OF THE COMMON BILE DUCT

H ENELL B CAVELL and G MALMFORS

From the Departments of P ediatrics and Paediatric Surgery University Hospital Lund Sweden

ABSTRACT Enell H Cavell B and Malmfors G (Departments of Paediatrics and Paediatric Surgery University Hospital Lund Sweden) Spontaneous perforation of the common bile duct Acta Paediatr Scand 68 625 1979 —An 18-week-old baby boy suffered an episode of acute abdominal symptoms followed by a silent period with mild obstructive jaundice abdominal distension and failure to thrive During the clinical work up he deteriorated suddenly with progressive abdominal distension and ascites At laparotomy a perforation of the common bile duct with bile peritonitis was found Spontaneous perforation of the common bile duct is seldom listed as a cause of obstructive jaundice The clinical picture is characteristic Awareness of this diagnosis may help to avoid time consuming and unnecessary investigations and to lead to surgical treatment in good time

KEY WORDS Obstructive jaundice ascites bile peritonitis infancy

Spontaneous perforation of the common bile duct is seldom discussed as a cause of obstructive jaundice in the paediatric literature (6 7) Lilly et al (5) however state that this disease is second only to biliary atresia as a cause of surgical jaundice in the newborn Until 1974 about 50 cases had been reported most of them in the surgical literature (5) The aim of this paper is to draw attention to this disease and to report a case with a protracted course

CASE HISTORY

An 18 week-old boy was referred to a local hospital because of abdominal pains irritability and anorexia Pregnancy delivery and neonatal period had been uneventful On admission the abdomen was distended and tender The stool were pale and the urine dark The emergency examination excluded an intestinal obstruction

The initial symptoms subsided gradually and the boy was referred ? weeks later to the Paediatric Department University Hospital His general condition was then fairly good Physical examination revealed a normally developed slightly jaundiced baby with moderate abdominal distension During the following weeks his general clinical condition further improved and his appetite returned His weight however was stationary and the stools were still light-coloured

…atory investigation showed a conjugated hyperbilirubinemia around 50 μmol/l Alkaline phosphatases 18 μkatal/l glutamyltransferase 4 8 μkatal/l aminotransferases normal prothrombin time 32 s urobilinogen/urine negative Serum proteins including immunoglobulins were normal Investigations for metabolic endocrine or infectious causes of the illness turned out normal

Abdominal plain films taken early in the course showed slightly dilated bowels A barium enema of the colon and an intravenous pyelography gave normal results

A choledochal cyst was suspected as a cause of the boy s stationary and mild cholestasis At intravenous cholegraphy the gallbladder appeared and emptied normally but the bile ducts were not visualized In order to exclude an expansivity close to the bile duct causing intermittent obstruction ultrasonic echogram and X ray of the stomach and upper small intestine were performed The patient deteriorated quickly however with marked abdominal distension and signs of ascites The X ray showed wide intestinal loops slow passage and ascites but no signs of tumour dislocating the viscera The ultrasonic echogram was negative The boy was referred for emergency exploratory laparotomy in the department of paediatric surgery

On admission to this department the patient was in a rather poor general condition Preoperatively he was given proper amounts of fluid electrolytes and plasma At laparotomy nearly one litre of bile stained fluid was emptied from the abdominal cavity before the bile ducts were exposed An attempt to make a cholangiogram through the gallbladder was not successful The choledochal duct was dissected and choledochotomy was performed The lumen of the choledochal duct was normal whereas the external diameter was more than 1 cm due to a peritonitic swelling A catheter was passed to the

CASE REPORT

SCLERODERMA WITH MASSIVE REGIONAL LYMPHADENOPATHY

S E TANGSRUD D SKYBERG and TOVE EEG LARSEN

From the Department of Paediatrics Sentralsjukehuset i Aust Agder Arendal Norway and the Department of Pathology Rikshospitalet Oslo Norway

ABSTRACT Tangsrud S E Skyberg D and Larsen Tove Eeg (Department of Paediatrics Sentralsjukehuset i Aust Agder Arendal Norway and the Department of Pathology Rikshospitalet Oslo Norway) Case Report Scleroderma with massive regional lymphadenopathy Acta Paediatr Scand 68 627 1979 —Scleroderma in a two-year-old boy with gross enlargement of the right inguinal lymph nodes as an early sign is reported Repeated lymph node biopsies revealed non characteristic reactive changes and hyperplasia but eventually histological examinations of skin and muscle from the right leg were diagnostic The effect of one year of D-penicillamine therapy is briefly mentioned

KEY WORDS Scleroderma lymphadenopathy D-penicillamine

Scleroderma is a connective tissue disease of unknown etiology It is rare in childhood and may involve the skin gastrointestinal tract heart lung and kidney There seems to be no sharp distinction between the focal form morphoea and the diffuse systemic disease (2 3) Raynaud s phenomenon positive antinuclear factor LE cells and raised erythrocyte sedimentation rate are associated with poor prognosis (2)

We present a case with regional lymphadenopathy as the initial sign

CASE REPORT

A previously healthy year-old boy was admitted because of enlargement of the inguinal lymph nodes and episodic pain in the right leg

The parents noticed the swelling in the groin 8 weeks prior to admission and 3 weeks later the mother also noted skin changes in the right leg Initially there were small red scaling patches on the skin later these patches became stretched and band like along the length of the leg

On admission his general condition was good General lymphadenopathy was absent Liver and spleen were not increased in size There was marked enlargement of the right inguinal lymph nodes which were firm tender and adherent to each other and to deeper structures Areas of increased pigmentation as well as areas with lack of pigmentation were noted mainly in the gluteal region perineum and the medial surface of the right leg The skin was thin atrophic and partly adherent to the underlying structures which were indurated Muscular wasting of the right calf and a 10–15 contracture in the right knee were present A telangiectatic area about 5 mm in diameter which had appeared during the first weeks of illness was noted on the right cheek Due to pain related to the lymph nodes he was almost unable to walk

Investigations

Hb 116 g/l ESR 25 mm/hr WBC 19.0×10^9/l with lymphocytic dominance platelets 88.0×10^9/l Serum ASAT 103 U/l (normal range 10–40 U/l) Serum ALAT 78 U/l (normal range 10–40 U/l) Gamma GT OCT LDH CPK and creatinine were all normal

The serum fractions of IgA IgM and IgE were normal while IgG was slightly elevated (16 1 g/l) Serological tests for ornithosis toxoplasmosis salmonellosis brucellosis streptococcosis mononucleosis and Epstain Barr virus were negative A PPD test was negative A negative LE preparation a positive test for antinuclear antibodies (titre 3) and a strongly positive anti DNA denaturated factor were found Serum complement C_3 and C ECG and respiratory function tests were within normal limits X ray examinations of chest oesophagus stomach and the skeleton were normal

A lymph node biopsy from the enlarged glands in the right inguinal region was performed the day after admission Three weeks afterwards another lymph node biopsy from the same region was performed together with a skin and muscle biopsy from the affected area of the right calf Both lymph node biopsies showed reactive changes and hyperplasia A skin biopsy from the lower extremity

duodenum proving passage. A leak was found a few millimetres distal of the cystic duct on the right lateral surface of the choledochal duct. The leak was oversewn and a choledochal drain was left in place. The area was thoroughly drained.

The postoperative course was quite uneventful. A cholangiogram on the seventh day was normal. A few months post-operatively the child was healthy with normal liver function tests.

DISCUSSION

The clinical features presented by our patient agree well with those reported as characteristic in the literature (2, 4, 5). The infants usually present with symptoms at the age of one week to 3 months. Initial signs include mild fluctuating jaundice, pale or acholic stools, dark urine, *irritability, vomiting and failure to thrive*. There is a progressive abdominal distension which is sometimes accompanied by bile staining of hydroceles, inguinal herniae or of the abdominal wall. The mild hyperbilirubinemia together with acholic stools indicate the correct diagnosis. The normal amino transferases are helpful in differentiating from hepatitis. The ^{131}I Rose Bengal test may establish the preseence of a biliary leak (1, 2).

A protracted course of the disease, as observed in the case here reported, seems to be the most usual one, but an acute mode of presentation is sometimes seen (4).

The symptoms and the time of onset are helpful in distinguishing the disease from other causes of obstructive jaundice in infancy, such as biliary atresia and choledochal cyst (3).

It has been suggested that spontaneous perforation of the common bile duct and choledochal cyst are different manifestations of a common developmental aberration in ductal embryogenesis (5).

In many cases of spontaneous perforation of the common bile duct the operative finding is an inflammatory sac, which has been misinterpreted as a ruptured choledochal cyst. This may lead to an inadequate operative management with lethal outcome (5). We think that such a sac was initially formed in our patient after which his condition improved. The sac then ruptured and gave rise to the alarming signs of bile peritonitis.

Different operative procedures have been suggested, from simple drainage to choledochal resections with hepatico-enteric anas*tomosis. It seems as the simple* drainage procedure is the method of choice (4, 5).

Spontaneous perforation of the common bile duct is a rare disease. On the other hand an awareness of the condition is important as im*mediate surgical treatment is indicated.*

REFERENCES

1. Hansen R, Wasnich R, De Vries P & Sunshine P. Bile ascites in infancy. Diagnosis with ^{131}I Rose Bengal. *J Pediatr 84*: 719, 1974.
2. Howard E, Johnston D & Mowat A. Spontaneous perforation of common bile duct in infants. *Arch Dis Child 51*: 883, 1976.
3. Kobayashi A & Ohbe Y. Choledochal cyst in infancy and childhood. *Arch Dis Child 52*: 121, 1977.
4. Lees W & Mitchell J. Bile peritonitis in infancy. *Arch Dis Child 41*: 188, 1966.
5. Lilly J, Weintraub W & Altman P. Spontaneous perforation of the extrahepatic bile ducts and bile peritonitis in infancy. *Surgery 75*: 664, 1974.
6. Mowat A. Persistent neonatal jaundice. *In* J T Harris (ed.) *Essentials of Pediatric Gastroenterology*. Churchill Livingstone, Edinburgh, London and New York 1977, p. 266.
7. Sass-Kortsak A. Management of young infants presenting with direct reacting hyperbilirubinemia. *Pediatr Clin N Am 21*: 777, 1974.

Submitted Nov. 13, 1978
Accepted Jan. 17, 1979

(H. E.) Department of Paediatrics
University Hospital
S-221 85 Lund
Sweden

CASE REPORT

SCLERODERMA WITH MASSIVE REGIONAL LYMPHADENOPATHY

S E TANGSRUD D SKYBERG and TOVE EEG LARSEN

From the Department of Paediatrics Sentralsjukehuset i Aust Agder Arendal Norway and the Department of Pathology Rikshospitalet Oslo Norway

ABSTRACT Tangsrud S E Skyberg D and Larsen Tove Eeg (Department of Paediatrics Sentralsjukehuset i Aust Agder Arendal Norway and the Department of Pathology Rikshospitalet Oslo Norway) Case Report Scleroderma with massive regional lymphadenopathy Acta Paediatr Scand 68 627 1979.—Scleroderma in a two-year-old boy with gross enlargement of the right inguinal lymph nodes as an early sign is reported Repeated lymph node biopsies revealed non characteristic reactive changes and hyperplasia but eventually histological examinations of skin and muscle from the right leg were diagnostic The effect of one year of D-penicillamine therapy is briefly mentioned

KEY WORDS Scleroderma lymphadenopathy D-penicillamine

Scleroderma is a connective tissue disease of unknown etiology it is rare in childhood and may involve the skin gastrointestinal tract heart lung and kidney There seems to be no sharp distinction between the focal form morphoea and the diffuse systemic disease (2 3) Raynaud s phenomenon positive antinuclear factor LE cells and raised erythrocyte sedimentation rate are associated with poor prognosis (2)

We present a case with regional lymphadenopathy as the initial sign

CASE REPORT

A previously healthy year-old boy was admitted because of enlargement of the inguinal lymph nodes and episodic pain in the right leg

The parent noticed the swelling in the groin 8 weeks prior to admission and 3 weeks later the mother also noted skin changes in the right leg Initially there were small red scaling patches on the skin later these patches became stretched and band like along the length of the leg

On admission his general condition was good General lymphadenopathy was absent Liver and spleen were not increased in size There was marked enlargement of the right inguinal lymph nodes which were firm tender and adherent to each other and to deeper structures Areas of increased pigmentation as well as areas with lack of pigmentation were noted mainly in the gluteal region perineum and the medial surface of the right leg The skin was thin atrophic and partly adherent to the under lying structures which were indurated Muscular wasting of the right calf and a 10–15 contracture in the right knee were present A telangiectatic area about 5 mm in diameter which had appeared during the first weeks of illness was noted on the right cheek Due to pain related to the lymph nodes he was almost unable to walk

Investigations

Hb 116 g/l ESR 75 mm/hr WBC 19 0×10⁹/l with lymphocytic dominance platelets 88 0×10⁹/l Serum ASAT 103 U/l (normal range 10–40 U/l) Serum ALAT 78 U/l (normal range 10–40 U/l) Gamma GT OCT LDH CPK and creatinine were all normal

The serum fractions of IgA IgM and IgE were normal while IgG was slightly elevated (16 1 g/l) Serological tests for ornithosis toxoplasmosis salmonellosis brucellosis streptococcosis mononucleosis and Epstain Barr virus were negative A PPD test was negative A negative LE preparation a positive test for antinuclear antibodies (titre 32) and a strongly positive anti DNA denaturated factor were found Serum complement C and C ECG and respiratory function tests were within normal limits X ray examinations of chest oesophagus stomach and the skeleton were normal

A lymph node biopsy from the enlarged glands in the right inguinal region was performed the day after admission Three weeks afterwards another lymph node biopsy from the same region was performed together with a skin and muscle biopsy from the affected area of the right calf Both lymph node biopsies showed reactive changes and hyperplasia A skin biopsy from the lower extremity

duodenum proving passage. A leak was found a few millimetres distal of the cystic duct on the right lateral surface of the choledochal duct. The leak was oversewn and a choledochal drain was left in place. The area was thoroughly drained.

The postoperative course was quite uneventful. A cholangiogram on the seventh day was normal. A few months postoperatively the child was healthy with normal liver function tests.

DISCUSSION

The clinical features presented by our patient agree well with those reported as characteristic in the literature (2, 4, 5). The infants usually present with symptoms at the age of one week to 3 months. Initial signs include mild fluctuating jaundice, pale or acholic stools, dark urine, irritability, vomiting and failure to thrive. There is a progressive abdominal distension which is sometimes accompanied by bile staining of hydroceles, inguinal herniae or of the abdominal wall. The mild hyperbilirubinemia together with acholic stools indicate the correct diagnosis. The normal amino transferases are helpful in differentiating from hepatitis. The [131]I Rose Bengal test may establish the preseence of a biliary leak (1, 2).

A protracted course of the disease, as observed in the case here reported, seems to be the most usual one, but an acute mode of presentation is sometimes seen (4).

The symptoms and the time of onset are helpful in distinguishing the disease from other causes of obstructive jaundice in infancy, such as biliary atresia and choledochal cyst (3).

It has been suggested that spontaneous perforation of the common bile duct and choledochal cyst are different manifestations of a common developmental aberration in ductal embryogenesis (5).

In many cases of spontaneous perforation of the common bile duct the operative finding is an inflammatory sac which has been misinterpreted as a ruptured choledochal cyst. This may lead to an inadequate operative management with lethal outcome (5). We think that such a sac was initially formed in our patient, after which his condition improved. The sac then ruptured and gave rise to the alarming signs of bile peritonitis.

Different operative procedures have been suggested, from simple drainage to choledochal resections with hepatico-enteric anastomosis. It seems as the simple drainage procedure is the method of choice (4, 5).

Spontaneous perforation of the common bile duct is a rare disease. On the other hand an awareness of the condition is important as immediate surgical treatment is indicated.

REFERENCES

1. Hansen R, Wasnich R, De Vries P & Sunshine P. Bile ascites in infancy. Diagnosis with [131]I Rose Bengal. *J Pediatr 84*: 719, 1974.
2. Howard E, Johnston D & Mowat A. Spontaneous perforation of common bile duct in infants. *Arch Dis Child 51*: 883, 1976.
3. Kobayashi A & Ohbe Y. Choledochal cyst in infancy and childhood. *Arch Dis Child 52*: 121, 1977.
4. Lees W & Mitchell J. Bile peritonitis in infancy. *Arch Dis Child 41*: 188, 1966.
5. Lilly J, Weintraub W & Altman P. Spontaneous perforation of the extrahepatic bile ducts and bile peritonitis in infancy. *Surgery 75*: 664, 1974.
6. Mowat A. Persistent neonatal jaundice. In J. T. Harris (ed.) *Essentials of Pediatric Gastroenterology*. Churchill Livingstone, Edinburgh, London and New York 1977, p. 266.
7. Sass-Kortsak A. Management of young infants presenting with direct reacting hyperbilirubinemia. *Pediatr Clin N Am 21*: 777, 1974.

Submitted Nov. 13, 1978
Accepted Jan. 17, 1979

(H. E.) Department of Paediatrics
University Hospital
S-221 85 Lund
Sweden

Acta Pædiatr Scand 68 627–628 1979

CASE REPORT

SCLERODERMA WITH MASSIVE REGIONAL LYMPHADENOPATHY

S E TANGSRUD D SKYBERG and TOVE EEG LARSEN

From the Department of Paediatrics Sentralsjukehuset i Aust Agder Arendal Norway and the Department of Pathology Rikshospitalet Oslo Norway

ABSTRACT Tangsrud S E Skyberg D and Larsen To e Eeg (Department of Paediatrics Sentralsjukehuset i Aust Agder Arendal Norway and the Department of Pathology Rikshospitalet Oslo Norway) Case Report Scleroderma with massive regional lymphadenopathy Acta Paediatr Scand 68 627 1979.—Scleroderma in a two-year-old boy with gross enlargement of the right inguinal lymph nodes as an early sign is reported Repeated lymph node biopsies revealed non characteristic reactive changes and hyperplasia but eventually histological examinations of skin and muscle from the right leg were diagnostic The effect of one year of D-penicillamine therapy is briefly mentioned

KEY WORDS Scleroderma lymphadenopathy D-penicillamine

Scleroderma is a connective tissue disease of unknown etiology it is rare in childhood and may involve the skin gastrointestinal tract heart lung and kidney There seems to be no sharp distinction between the focal form morphoea and the diffuse systemic disease (2 3) Raynaud s phenomenon positive antinuclear factor LE cells and raised erythrocyte sedimentation rate are associated with poor prognosis (2)

We present a case with regional lymphadenopathy as the initial sign

CASE REPORT

A previously healthy year-old boy was admitted because of enlargement of the inguinal lymph nodes and episodic pain in the right leg

The parents noticed the swelling in the groin 8 weeks prior to admission and 3 weeks later the mother also noted skin changes in the right leg Initially there were small red scaling patches on the skin later these patches became stretched and band like along the length of the leg

On admission his general condition was good General lymph denopathy was absent Liver and spleen were not increased in size There was marked enlargement of the right inguinal lymph nodes which were firm tender and adherent to each other and to deeper structures Areas of increased pigmentation as well as areas with lack of pigmentation were noted mainly in the gluteal region perineum and the medial surface of the right leg The skin was thin atrophic and partly adherent to the under lying structures which were indurated Muscular wasting of the right calf and a 10–15 contracture in the right knee were present A telangiectatic area about 5 mm in diameter which had appeared during the first weeks of illness was noted on the right cheek Due to pain related to the lymph nodes he was almost unable to walk

Investigations

Hb 116 g/l ESR 75 mm/hr WBC 19.0×10^9/l with lymphocytic dominance platelets 88.0×10^9/l Serum ASAT 103 U/l (normal range 10–40 U/l) Serum ALAT 78 U/l (normal range 10–40 U/l) Gamma GT OCT LDH CPK and creatinine were all normal

The serum fractions of IgA IgM and IgE were normal while IgG was slightly elevated (16 1 g/l) Serological tests for ornithosis toxoplasmosis salmonellosis brucellosis streptococcosis mononucleosis and Epstain Barr virus were negative A PPD test was negative A negative LE preparation a positive test for antinuclear antibodies (titre 3) and a strongly positive anti DNA denaturated factor were found Serum complement C and C ECG and respiratory function tests were within normal limits X ray examinations of chest oesophagus stomach and the skeleton were normal

A lymph node biopsy from the enlarged glands in the right inguinal region was performed the day after admission Three weeks afterwards another lymph node biopsy from the same region was performed together with a skin and muscle biopsy from the affected area of the right calf Both lymph node biopsies showed reactive changes and hyperplasia A skin biopsy from the lower extremity



Fig 1 The patient's right leg after 6 months of illness

showed dermal fibrosis and fibrotic subcutaneous septa In the lower dermis and in the subcutaneous septa there were dilated blood vessels and a perivascular as well as diffuse lympho plasmocytic infiltration This picture was compatible with scleroderma

Treatment and subsequent course

Several authors have reported encouraging results with the use of D penicillamine in treatment of childhood scleroderma (4 5) Our patient received 150 mg D-penicillamine every second day During an observation time of one year no side effects were observed

In addition he was given physiotherapy The skin has gradually improved and the induration of the deeper structures has become less firm The boy has complained of dysphagia and a barium X ray examination of the oesophagus revealed a probable dysfunction of the distal part of this organ His general condition is good and ESR has normalized

DISCUSSION

Reports on childhood scleroderma are few In some larger series (1 2 3) none of the patients were reported to have lymph node enlargement Dubich et al (1) found skin changes as presenting sign in 100% in a group of twelve children Joint pains contractures and Raynaud phenomena are also frequent (1 3)

Because of serological and clinical overlap specific diagnosis in childhood connective tissue diseases is often difficult Differential diagnoses include dermatomyositis juvenile rheumatoid arthritis and SLE

Thomson & Milne (5) reported a girl who when 12 years old presented with enlarged neck lymph nodes and a skin rash resembling erythema multiforme A lymph node biopsy showed no specific changes She developed arthritis and 4 years later scleroderma was diagnosed by a skin and muscle biopsy

The present case and the case reported by Thomson & Milne (5) draw attention to the fact that lymph node enlargement may be a prominent and early sign in childhood scleroderma and may precede cutaneous manifestations Biopsies of the affected lymph nodes are of little diagnostic value In the series of Dubich et al (1) the average time between appearance of symptoms and the final diagnosis was 2 years

Early skin and muscle biopsies should therefore be considered in any obscure cases of childhood connective tissue disease with skin or lymph node involvement The course in our patient may support the value of D-penicillamine in the treatment of scleroderma (4 5)

REFERENCES

1 Dubich I Sullivan D & Cassidy J T Scleroderma in the child *Pediatr 85* 6 770 1974
2 Goel K M & Shanks R A Scleroderma in childhood Report of five cases *Arch Dis Child 49* 86 1974
3 Kass H Hanson V & Patrick J Scleroderma in childhood *J Pediatr 68* 243 1966
4 Moynahan E J Penicillamine in the treatment of morphoea and keloid in children *Postgrad Med J 50* 39 1974
5 Thomson J & Milne J A Two years of penicillamine for progressive systemic sclerosis A case report *Postgrad Med J 50* 36 1974

Submitted July 11 1978
Accepted Jan 22 1979

(S E T) Department of Paediatrics
Ullevål Hospital
Oslo 1
Norway

Acta Pædiatr Scand 68 629–634 1979

CASE REPORT

NONKETOTIC HYPERGLYCINEMIA

Clinical Biochemical and Therapeutic Aspects

S KØLVRAA N J BRANDT and E CHRISTENSEN

From the Section of Clinical Genetics Department of Paediatrics and the Department of Neonatology University of Copenhagen Rigshospitalet Denmark

ABSTRACT Kølvraa S Brandt N J and Christensen E (Section of Clinical Genetics Department of Paediatrics and Department of Neonatology University of Copenhagen Rigshospitalet Copenhagen Denmark) Nonketotic hyperglycinemia Clinical biochemical and therapeutic aspects Acta Paediatr Scand 68 629 1979 —A patient exhibiting progressive cerebral depression from the first days of life is described The diagnosis of nonketotic hyperglycinemia was established on the typical clinical presentation elevated glycine concentrations in body fluids and diminished glycine cleavage activity in liver tissue A series of therapeutic trials including strychnine treatment was tried on this patient without apparent effect on the clinical course

KEY WORDS Nonketotic hyperglycinemia glycine cleavage activity strychnine treatment

Hyperglycinemia was first described as a syndrome by Childs et al (3) It is now possible on clinical and biochemical criteria to differentiate this syndrome into several distinct entities namely the disease presented in this paper nonketotic hyperglycinemia the ketotic forms propionic acidemia (1) methylmalonic acidemia (10) and β ketothiolase deficiency (5) and the newly published D-glyceric acidemia (2 7)

Nonketotic hyperglycinemia is clinically characterized by neonatal onset of hypotonia lethargy and seizures followed by severe mental retardation Biochemically it is characterized by elevated glycine concentrations in blood urine and spinal fluid and diminished activity of the glycine cleavage system in liver and brain (12)

Many therapeutic attempts have been tried to relieve the symptoms of nonketotic hyperglycinemia Most of these have aimed at lowering the glycine concentration in serum either by diet by administering compounds that form easily excretable conjugates with glycine or by donating C_1 fragments (11) Recently a more direct attempt to lower spinal fluid glycine has been tried by Krieger et al (6) namely insertion of a ventricular shunt Strychnine treatment has also been tried (4) This is based on the observation that the effect of glycine on synaptic transmission is contrary to the effect of strychnine especially on spinal centres (15)

The present communication describes the clinical and biochemical presentation of a case of nonketotic hyperglycinemia together with the therapeutic attempts that we have performed

CASE REPORT

The patient a boy is the first child of nonconsanguineous parents The mother has in a previous marriage given birth to a normal boy Pregnancy and delivery was uneventful Birth weight 3 600 g The child appeared normal at birth but from the second day of life he became increasingly listless From the fifth day insufficient respiration occurred which caused the patient to be transferred to the University Hospital Examination on admission revealed a severely hypotonic child with no spontaneous movements and no reactions to stimuli Artificial

41 79 4

Table 1. *Quantitative determination of serum and urine amino acids*

	Serum (µmol/l)		Urine (µmol/24 hrs)	
	Patient	Range in controls	Patient	Range in controls[b]
Aspartic acid	91	25–72	19	33–181
Threonine	149	69–125	47	4–125
Serine	435	103–212	102	5–241
Aspargine	69	37–51	59	5–108
Glutamic acid/glutamine	497	309–422	56	46–277
Proline	246	119–233	Trace	Trace
Glycine	1 226	160–282	4 894	166–495
Alanine	387	272–375	80	45–231
Valine	217	123–206	5	11–22
Cystine	31	28–38	9	Trace–11
Methionine	29	12–18	Trace	Trace–12
Isoleucine	85	30–57	Trace	Trace–9
Leucine	155	64–104	Trace	Trace–19
Tyrosine	87	37–52	29	Trace–71
Phenylalanine	54	35–54	8	Trace–56
Ornithine	146	62–92	Trace	Trace–79
Lysine	166	114–201	30	19–75
Histidine	97	58–88	211	117–686
Arginine	88	61–129	4	Trace–10

4 children 1–6 years old (2).
[b] 8 children 1–6 years old (2).
Trace <2 µmol/24 hrs.

respiration had to be instituted immediately after admission.

Laboratory investigations on admission. Routine laboratory investigations including haemoglobin, white blood cell count, thrombocytes, blood glucose and electrolytes were normal. The acid base status showed a moderate respiratory acidosis. No ketosis was present. Amino acid analysis of serum and urine both demonstrated marked elevation of glycine (Table 1). Spinal fluid was obtained in a period where the serum glycine concentration was stabilized around 500 µmol/l. At the day of sampling serum glycine was 524 µmol/l and spinal fluid glycine 187 µmol/l.

Further clinical course. Intensive glycine depleting therapy was given from the 8th to the 28th day of life (see "Initial therapeutic attempts"). This resulted in reestablishment of spontaneous respiration, but apart from this, no clinical improvement could be demonstrated. From the 10th day of life seizures resembling infantile myoclonic jerks occurred, which necessitated antiepileptic treatment. EEG at this time showed severe hypsarrhythmia. The further clinical course has been characterized by a convulsive disorder difficult to control (in spite of treatment with Clonazepam (Rivotril®) 1.2 mg/day and Phenobarbital 30 mg/day) and severe mental retardation. Physically, he has developed normally. Weight, length and head circumference have constantly been within the tenth percentile.

MATERIAL AND METHODS

Amino acids and organic acids were measured as previously described (7).

Glycine cleavage activity. At six months of age a surgical liver biopsy was obtained from the patient. The biopsy was immediately frozen and kept at −70°C. Glycine cleavage activity was measured according to Sato et al. (13). 25 mg liver tissue was homogenized and incubated with ^{14}C-1-glycine. Reaction mixture: 4.75 µmol glycine (specific activity: 0.6 mCi/mmol), 0.5 µmol tetrahydrofolic acid, 0.8 µmol pyridoxal phosphate, 2.5 µmol dithiotreitol, 0.8 µmol nicotinamid adenine-dinucleotide, 75 µmol Tris HCl (pH=8.1), 3 mg protein, total volume 1.0 ml.

After incubation for one hour the reaction was stopped by adding 0.2 ml sulphuric acid (5 mol/l). The liberated CO_2 was trapped on Protozol®, scintillation fluid added and ^{14}C activity counted in a liquid scintillation counter. Activity was expressed in nmol CO_2 produced/mg protein/hour. Protein was determined according to Lowry (9).

RESULTS

Amino acids. The amino acid examinations demonstrated an isolated accumulation of glycine in body fluids both on admission (Table 1) and on several later occasions when amino acids were investigated in connection with therapeutic attempts. The ratio of spinal fluid glycine to serum glycine was 0.36.

Organic acids. Low molecular weight organic acids in the urine were examined by gas

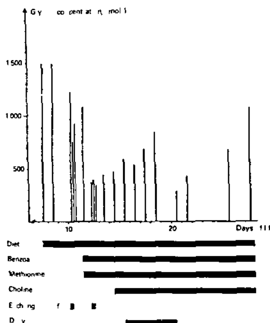


Fig 1 Course of serum glycine concentration during the initial therapeutic attempts

chromatography as trimethylsilyl derivatives and as free volatile acids No accumulation of propionic acid methylmalonic acid 2 methyl 3 hydroxybutyric acid 2 methyl 3 oxo-butyric acid or D-glyceric acid could be demonstrated

Glycine cleavage In preliminary experiments it was ascertained that the glycine cleavage reaction rate increased linearly with the protein concentration and that the reaction proceeded linearly with time for at least one hour

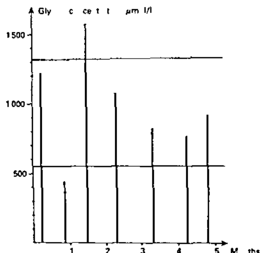


Fig 2 Spontaneous fluctuation in serum glycine concentration during a 5 month period The horizontal lines indicate range defined as mean serum glycine concentration ± S D

Tissue samples from two autoptic livers obtained 8 and 12 hours post mortem and one surgical liver biopsy were used as controls The glycine cleavage activities found are shown in Table 2 together with the diagnoses of the children

Table 2 *Glycine cleavage activity in liver tissue*

	Diagnosis	Glycine cleavage (nmol/mg protein/hour)
Patient	Nonketotic hypergly cinemia	0 095
Control A	Mild hepatic stasis (surgical biopsy)	0 570
Control B	Cong heart disease (autoptic biopsy)	0 9?0
Control C	Cong heart disease (autoptic biopsy)	1 050

THERAPEUTIC ATTEMPTS

Initial therapeutic attempts The diagnosis nonketotic hyperglycinemia was established on the eighth day of life and the following therapeutic steps were taken (Fig 1) Breast milk was substituted by a mixture containing per liter 25 g glycine serine and proline free mixture of amino acids salts and vitamins (Maizena Haus Hamburg) 30 g glucose 30 g fructose 350 ml Intralipid* ?00 ml per day of this mixture was given initially gradually increasing to 700 ml per day at day 25 In addition the child received the usual vitamin supplement for his age On the first day of therapy an exchange transfusion was performed and medication was started with benzoic acid ?00 mg/kg/day and methionine 150 mg/kg/day

Two days later another exchange transfusion was performed and treatment with choline bitartrate 85 mg/kg/day was instituted Finally from the 16th day to the ?0th day of life a continuous peritoneal dialysis was performed During this treatment the general condition improved only slightly although at the end of the period the patient was capable of unassisted breathing He was however still extremely hypotonic and showed only very weak reactions to stimuli

Table 1 *Quantitative determination of serum and urine amino acids*

	Serum (μmol/l)		Urine (μmol/24 hrs)	
	Patient	Range in controls	Patient	Range in controls[a]
Aspartic acid	91	25– 72	19	33–181
Threonine	149	69–125	47	4–125
Serine	435	103–212	102	5–241
Aspargine	69	37– 51	59	5–108
Glutamic acid/glutamine	497	309–422	56	46–277
Proline	246	119–233	Trace	Trace
Glycine	1 226	160–282	4 894	166–495
Alanine	387	272–375	80	45–231
Valine	217	123–206	5	11– 22
Cystine	31	28– 38	9	Trace–11
Methionine	29	12– 18	Trace	Trace–12
Isoleucine	85	30– 57	Trace	Trace–9
Leucine	155	64–104	Trace	Trace–19
Tyrosine	87	37– 52	29	Trace–71
Phenylalanine	54	35– 54	8	Trace–56
Ornithine	146	62– 92	Trace	Trace–79
Lysine	166	114–201	30	19– 75
Histidine	97	58– 88	211	117–686
Arginine	88	61–129	4	Trace–10

4 children 1–6 years old (2)
[b] 8 children 1–6 years old (2)
Trace <2 μmol/24 hrs

respiration had to be instituted immediately after admission

Laboratory investigations on admission Routine laboratory investigations including haemoglobin white blood cell count thrombocytes blood glucose and electrolytes were normal The acid base status showed a moderate respiratory acidosis No ketosis was present Amino acid analysis of serum and urine both demonstrated marked elevation of glycine (Table 1) Spinal fluid was obtained in a period where the serum glycine concentration was stabilized around 500 μmol/l At the day of sampling serum glycine was 524 μmol/l and spinal fluid glycine 187 μmol/l

Further clinical course Intensive glycine depleting therapy was given from the 8th to the 28th day of life (see Initial therapeutic attempts) This resulted in re establishment of spontaneous respiration but apart from this no clinical improvement could be demonstrated From the 10th day of life seizures resembling infantile myoclonic jerks occurred which necessitated antiepileptic treatment EEG at this time showed severe hypsarrhythmia The further clinical course has been characterized by a convulsive disorder difficult to control (in spite of treatment with *Clonazepam (Rivotril®) 1 2 mg/* day and Phenobarbital 30 mg/day) and severe mental retardation Physically he has developed normally Weight length and head circumference have constantly been within the tenth percentile

MATERIAL AND METHODS

Amino acids and organic acids were measured as previously described (7)

Glycine cleavage activity At six months of age a surgical liver biopsy was obtained from the patient The biopsy was immediately frozen and kept at − 0°C Glycine cleavage activity was measured according to Sato et al (13) 25 mg liver tissue was homogenized and incubated with [14]C 1 glycine Reaction mixture 4 75 μmol glycine (specific activity 0 6 mCi/mmol) 0 5 μmol tetrahydrofolic acid 0 8 μmol pyridoxal phosphate 2 5 μmol dithiotreitol 0 8 μmol nicotinamid adenine-dinucleotide 75 μmol Tris HCl (pH=8 1) 3 mg protein total volume 1 0 ml

After incubation for one hour the reaction was stopped by adding 0 2 ml sulphuric acid (5 mol/l) The liberated CO_2 was trapped on Protozol® scintillation fluid added and [14]C activity counted in a liquid scintillation counter Activity was expressed in nmol CO produced mg protein/hour Protein was determined according to Lowry (9)

RESULTS

Amino acids The amino acid examinations demonstrated an isolated accumulation of glycine in body fluids both on admission (Table 1) and on several later occasions when amino acids were investigated in connection with therapeutic attempts The ratio of spinal fluid glycine to serum glycine was 0 36

Organic acids Low molecular weight organic acids in the urine were examined by gas

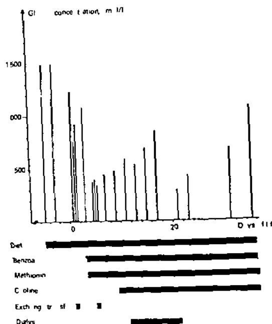


Fig 1 Course of serum glycine concentration during the initial therapeutic attempts

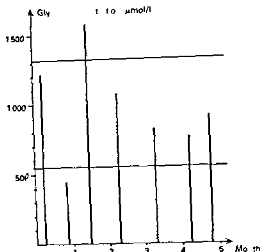


Fig 2 Spontaneous fluctuation in serum glycine concentration during a 5 month period The horizontal lines indicate range defined as mean serum glycine concentration ± S D

chromatography as trimethylsilyl derivatives and as free volatile acids No accumulation of propionic acid methylmalonic acid 2 methyl 3 hydroxybutyric acid 2 methyl 3 oxo-butyric acid or D-glyceric acid could be demonstrated

Glycine cleavage In preliminary experiments it was ascertained that the glycine cleavage reaction rate increased linearly with the protein concentration and that the reaction proceeded linearly with time for at least one hour

Tissue samples from two autoptic livers obtained 8 and 12 hours post mortem and one surgical liver biopsy were used as controls The glycine cleavage activities found are shown in Table 2 together with the diagnoses of the children

Table 2 *Glycine cleavage activity in liver tissue*

	Diagnosis	Glycine cleavage (nmol/mg protein/hour)
Patient	Nonketotic hypergly cinemia	0 095
Control A	Mild hepatic stasis (surgical biopsy)	0 570
Control B	Cong heart disease (autoptic biopsy)	0 9 0
Control C	Cong heart disease (autoptic biopsy)	1 050

THERAPEUTIC ATTEMPTS

Initial therapeutic attempts The diagnosis nonketotic hyperglycinemia was established on the eighth day of life and the following therapeutic steps were taken (Fig 1) Breast milk was substituted by a mixture containing per liter 25 g glycine serine and proline free mixture of amino acids salts and vitamins (Maizena Haus Hamburg) 30 g glucose 30 g fructose 350 ml Intralipid® 400 ml per day of this mixture was given initially gradually increasing to 700 ml per day at day 25 In addition the child received the usual vitamin supplement for his age On the first day of therapy an exchange transfusion was performed and medication was started with benzoic acid 700 mg/kg/day and methionine 150 mg/kg/day

Two days later another exchange transfusion was performed and treatment with choline bitartrate 85 mg/kg/day was instituted Finally from the 16th day to the 20th day of life a continuous peritoneal dialysis was performed During this treatment the general condition improved only slightly although at the end of the period the patient was capable of unassisted breathing He was however still extremely hypotonic and showed only very weak reactions to stimuli

Table 1. *Quantitative determination of serum and urine amino acids*

	Serum (μmol/l)		Urine (μmol/24 hrs)	
	Patient	Range in controls	Patient	Range in controls[a]
Aspartic acid	91	25– 72	19	33–181
Threonine	149	69–125	47	4–125
Serine	435	103–212	102	5–241
Aspargine	69	37– 51	59	5–108
Glutamic acid/glutamine	497	309–422	56	46–277
Proline	246	119–233	Trace	Trace
Glycine	1 276	160–282	4 894	166–495
Alanine	387	272–375	80	45–231
Valine	217	123–206	5	11– 22
Cystine	31	28– 38	9	Trace–11
Methionine	29	12– 18	Trace	Trace–12
Isoleucine	85	30– 57	Trace	Trace–9
Leucine	155	64–104	Trace	Trace–19
Tyrosine	87	37– 52	29	Trace–71
Phenylalanine	54	35– 54	8	Trace–56
Ornithine	146	62– 92	Trace	Trace–79
Lysine	166	114–201	30	19– 75
Histidine	97	58– 88	211	117–686
Arginine	88	61–129	4	Trace–10

4 children 1–6 years old (2)
[a] 8 children 1–6 years old (2)
Trace <2 μmol/24 hrs

respiration had to be instituted immediately after admission.

Laboratory investigations on admission. Routine laboratory investigations including haemoglobin, white blood cell count, thrombocytes, blood glucose and electrolytes were normal. The acid base status showed a moderate respiratory acidosis. No ketosis was present. Amino acid analysis of serum and urine both demonstrated marked elevation of glycine (Table 1). Spinal fluid was obtained in a period where the serum glycine concentration was stabilized around 500 μmol/l. At the day of sampling serum glycine was 524 μmol/l and spinal fluid glycine 187 μmol/l.

Further clinical course. Intensive glycine depleting therapy was given from the 8th to the 28th day of life (see "Initial therapeutic attempts"). This resulted in reestablishment of spontaneous respiration, but apart from this, no clinical improvement could be demonstrated. From the 10th day of life seizures resembling infantile myoclonic jerks occurred, which necessitated antiepileptic treatment. EEG at this time showed severe hypsarrhythmia. The further clinical course has been characterized by a convulsive disorder difficult to control (in spite of treatment with Clonazepam (Rivotril®) 1.2 mg/day and Phenobarbital 30 mg/day) and severe mental retardation. Physically, he has developed normally. Weight, length and head circumference have constantly been within the tenth percentile.

MATERIAL AND METHODS

Amino acids and organic acids were measured as previously described (7).

Glycine cleavage activity. At six months of age a surgical liver biopsy was obtained from the patient. The biopsy was immediately frozen and kept at −80°C. Glycine cleavage activity was measured according to Sato et al. (13). 25 mg liver tissue was homogenized and incubated with ^{14}C-1-glycine. Reaction mixture: 4.75 μmol glycine (specific activity 0.6 mCi/mmol), 0.5 μmol tetrahydrofolic acid, 0.8 μmol pyridoxal phosphate, 2.5 μmol dithiotreitol, 0.8 μmol nicotinamid-adenine-dinucleotide, 75 μmol Tris-HCl (pH=8.1), 3 mg protein, total volume 1.0 ml.

After incubation for one hour the reaction was stopped by adding 0.2 ml sulphuric acid (5 mol/l). The liberated CO_2 was trapped on Protozol® scintillation fluid added, and ^{14}C activity counted in a liquid scintillation counter. Activity was expressed in nmol CO_2 produced/mg protein/hour. Protein was determined according to Lowry (9).

RESULTS

Amino acids. The amino acid examinations demonstrated an isolated accumulation of glycine in body fluids both on admission (Table 1) and on several later occasions when amino acids were investigated in connection with therapeutic attempts. The ratio of spinal fluid glycine to serum glycine was 0.36.

Organic acids. Low molecular weight organic acids in the urine were examined by gas

The prognosis of nonketotic hyperglycinemia is extremely poor About 10% of the patients die in the neonatal period and the surviving patients suffer from severe cerebral damage (11) Several therapeutic trials have therefore been performed (11) Initially diets were used either protein free or free of glycine serine and proline

Later medication was tried with compounds that react with glycine followed by elimination of the conjugate such as benzoic acid and acetylsalicylic acid In recent years still another approach has been proposed namely to donate C_1 fragments This is based on the assumption that there is a shortage of C_1 fragments due to deficiency of the glycine cleavage activity The C_1 fragments should then diminish the glycine pool by transforming glycine to serine Of possible C_1-donors methionine choline and leucoverine have been tried Varying results have been reported but generally no effect on the clinical course and only weak and temporary effects on the serum glycine concentration have been obtained with these treatments

Because of the early diagnosis in our patient we decided to make a massive therapeutic effort in the neonatal period in order to see if mental retardation could be prevented As shown in Table 1 rapid lowering of serum glycine was obtained It seems however that the effect was achieved only by exchange transfusion and dialysis since serum glycine concentrations immediately started to rise after termination of these finally reaching pretreatment values in spite of diet and continuous treatment with benzoate methionine and choline

It is therefore likely that neither benzoate nor methionine nor choline have sufficient glycine depleting effect to be of therapeutic benefit This impression was further confirmed in the systematic trials where no significant effect could be demonstrated after treatment for 10 days with any of these compounds

A major reason for the almost total lack of clinical response on the normalisation of serum glycine concentration induced by exchange transfusion and dialysis is most likely that the symptoms are not caused by the elevated serum glycine They probably result from a raised spinal fluid glycine concentration especially in the synaptical cleft where glycine exerts its neurophysiological effect (14) The concentration here is normally extremely low (about 25 μmol/l) due to both a high and a low affinity transport system which removes glycine by transporting it against gradients both into the cells and into the blood stream (8) The spinal fluid glycine concentration in nonketotic hyperglycinemia is disproportionately high This is probably due to the lack of cerebral glycine cleavage activity which causes intracerebral and spinal fluid glycine accumulation When the spinal fluid glycine concentration reaches a certain level it results in saturation of the transport systems with further rapid raising of the spinal fluid glycine concentration as a result When the patient has reached this state lowering the serum glycine concentration will probably be almost completely ineffective since only a diminution of the cerebrospinal glycine pool extensive enough to create a concentration gradient direct away from the cerebrospinal room would re establish the transport systems within reasonable time

Therapeutic trials in nonketotic hyperglycinemia have therefore in the last few years been centered more on the cerebral mechanism than on lowering the serum concentration The first attempts along these lines were made by Krieger et al (6) who implanted a ventricular shunt in order to wash out the spinal fluid glycine more effectively Unfortunately the patient was only followed 5 weeks postoperatively so the effect of this treatment is uncertain More recently Gitzelman et al (4) have tried strychnine treatment with excellent effect on muscular tonus These promising results prompted us to try this treatment in our patient We could not however demonstrate any effect but this might be due to the fact

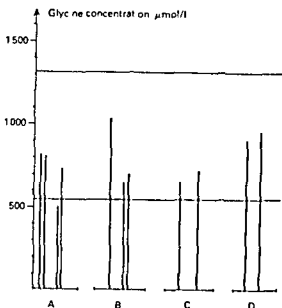


Fig 3 Serum glycine concentration during therapeutic attempts (*A*) benzoic acid 200 mg/kg/day (*B*) methionine 150 mg/kg/day (*C*) choline 85 mg/kg/day (*D*) benzoic acid 200 mg/kg/day + methionine 150 mg/kg/day Prior to each therapeutic attempt the serum glycine concentration was measured (first set of bars) Treatment was then given for 10 days On the last day of treatment the serum glycine concentration was measured again (second set of bars) The horizontal lines indicate range during periods without treatment

The effect on the glycine concentration in serum of the various forms of treatment is shown in Fig 1

Longterm spontaneous fluctuations in glycine concentrations When it was apparent that massive mental retardation was inevitable all treatments were withdrawn and benzoic acid methionine and choline were tried separately for ten days each to further elucidate their effects on the hyperglycinemia

*Prior to such a systematic investigation it was con*sidered necessary to clarify the magnitude of spontaneous fluctuations in the serum glycine concentration since these might bias the results

In order to achieve this serum samples collected more than 3 weeks after termination of any therapy were ob*tained over a* period of five months The glycine concentrations of these samples are shown in Fig 2 (mean glycine concentration ±S D 921±389 µmol/l)

Therapeutic attempts with benzoate methionine and choline All therapeutic attempts were performed in a similar manner when the patient was 1–2 years old *Initially the serum* glycine concentration was measured either as a single or as a double determination on two consecutive days Treatment was given for ten days with either benzoate 200 mg/kg/day methionine 150 mg/kg/day choline 85 mg/kg/day or the combination of benzoate 200 mg/kg/day and methionine 150 mg/kg/day At the end of each 10-day period serum glycine concentration was again measured either as a single or as a double determination The results of these investigations are shown in Fig 3

A certain effect of benzoate (3*A*) and methionine (3*B*) was apparent an effect however that could not be reproduced when giving the two compounds together (3*D*) The reason for this inconsistency is most likely to be the substantial intraindividual fluctuations in serum glycine concentration that are pictured in Fig 2 Medication with choline had no effect on the serum glycine concentration (3*C*)

No clinical improvement could be demonstrated during any of these trials neither on tonus nor on number of seizures

Strychnine treatment Treatment with strychnine nitrate 0 3 mg/kg/day was tried when the patient was 6 months old but neither improved tonus nor diminished number of seizures was achieved

DISCUSSION

The clinical picture with elevated concentrations of glycine in body fluids and absence of increased excretion of organic acids confirmed the diagnosis nonketotic hyperglycinemia It was further supported by the demonstration of diminished glycine cleavage activity in liver tissue As shown in Table 2 a higher activity was found in autoptic material compared to the surgical liver biopsy The significance of this is at present unclear but since the patient possessed only 20% of the activity in fresh liver tissue we believe it is safe to conclude that diminished glycine cleavage activity was present

Additional support for the diagnosis was achieved by the finding that the ratio of glycine concentration in spinal fluid to that in serum was 0 36 This ratio is normally 0 02 As pointed out by Perry et al (12) elevation of this ratio may indicate nonketotic hyperglycinemia where ratios about 0 2 have been found

The basic defect in this disorder is considered to be the diminished glycine cleavage activity which has been demonstrated in all cases so far investigated In liver tissue activities between 10 and 50% of normal have been found (12) In brain tissue the glycine cleavage activity is normally about 50% of the liver activity while in patients with nonketotic hyperglycinemia no activity could be demonstrated (12)

The prognosis of nonketotic hyperglycinemia is extremely poor About 10% of the patients die in the neonatal period and the surviving patients suffer from severe cerebral damage (11) Several therapeutic trials have therefore been performed (11) Initially diets were used either protein free or free of glycine serine and proline

Later medication was tried with compounds that react with glycine followed by elimination of the conjugate such as benzoic acid and acetylsalicylic acid In recent years still another approach has been proposed namely to donate C_1 fragments This is based on the assumption that there is a shortage of C_1 fragments due to deficiency of the glycine cleavage activity The C_1 fragments should then diminish the glycine pool by transforming glycine to serine Of possible C_1 donors methionine choline and leucoverine have been tried Varying results have been reported but generally no effect on the clinical course and only weak and temporary effects on the serum glycine concentration have been obtained with these treatments

Because of the early diagnosis in our patient we decided to make a massive therapeutic effort in the neonatal period in order to see if mental retardation could be prevented As shown in Table 1 rapid lowering of serum glycine was obtained It seems however that the effect was achieved only by exchange transfusion and dialysis since serum glycine concentrations immediately started to rise after termination of these finally reaching pretreatment values in spite of diet and continuous treatment with benzoate methionine and choline

It is therefore likely that neither benzoate nor methionine nor choline have sufficient glycine depleting effect to be of therapeutic benefit This impression was further confirmed in the systematic trials where no significant effect could be demonstrated after treatment for 10 days with any of these compounds

A major reason for the almost total lack of clinical response on the normalisation of serum glycine concentration induced by exchange transfusion and dialysis is most likely that the symptoms are not caused by the elevated serum glycine They probably result from a raised spinal fluid glycine concentration especially in the synaptical cleft where glycine exerts its neurophysiological effect (14) The concentration here is normally extremely low (about 25 µmol/l) due to both a high and a low affinity transport system which removes glycine by transporting it against gradients both into the cells and into the blood stream (8) The spinal fluid glycine concentration in nonketotic hyperglycinemia is disproportionately high This is probably due to the lack of cerebral glycine cleavage activity which causes intracerebral and spinal fluid glycine accumulation When the spinal fluid glycine concentration reaches a certain level it results in saturation of the transport systems with further rapid raising of the spinal fluid glycine concentration as a result When the patient has reached this state lowering the serum glycine concentration will probably be almost completely ineffective since only a diminution of the cerebrospinal glycine pool extensive enough to create a concentration gradient direct away from the cerebrospinal room would re establish the transport systems within reasonable time

Therapeutic trials in nonketotic hyperglycinemia have therefore in the last few years been centered more on the cerebral mechanism than on lowering the serum concentration The first attempts along these lines were made by Krieger et al (6) who implanted a ventricular shunt in order to wash out the spinal fluid glycine more effectively Unfortunately the patient was only followed 5 weeks post operatively so the effect of this treatment is uncertain More recently Gitzelman et al (4) have tried strychnine treatment with excellent effect on muscular tonus These promising results prompted us to try this treatment in our patient We could not however demonstrate any effect but this might be due to the fact

that the treatment was initiated when he was 26 months old and already severely brain damaged

ACKNOWLEDGEMENT

This work was supported by the Danish Medical Research Council

REFERENCES

1 Ando T & Nyhan W L Propionic acidemia and the ketotic hyperglycinemia syndrome *In* W L Nyhan (ed) *Heritable disorders of amino acid metabolism* John Wiley & Sons New York 1974
2 Brandt N J Rasmussen K Brandt S Kolvraa S & Schønheyder F D-glyceric acidemia and nonketotic hyperglycinemia *Acta Paediatr Scand 65* 17 1976
3 Childs B Nyhan W L Borden M Bard L & Cook R E Idiopathic hyperglycinemia and hyperglycinuria A new disorder of amino acid metabolism *Pediatrics 27* 522 1961
4 Gitzelmann R Cuenod M Otten A Steinmann B & Dumermuth G Nonketotic hyperglycinemia treated with strychnine *Pediatr Res 11* 1016 1977
5 Hillman R E & Keating J P *β* ketothiolase deficiency as a cause of the ketotic hyperglycinemia syndrome *Pediatrics 53* 221 1974
6 Krieger J Winbaum E S & Eisenbrey A B Cerebro-spinal fluid glycine in nonketotic hyperglycinemia Effect of treatment with sodium benzoate and a ventricular shunt *Metabolism 26* 517 1977
7 Kolvraa S Rasmussen K & Brandt N J D-glyceric acidemia biochemical studies of a new syndrome *Pediatr Res 10* 825 1976
8 Lajtha A Amino acid transport in the brain in vivo and in vitro *In* G E W Wolstenholm & D W Fitzsimmons (eds) *Aromatic amino acids in the brain* Ciba Foundation Symp 22 p 25 Elsevier New York 1974
9 Lowry O H Rosebrough N J Farr A L & Randall R J *J Biol Chem 193* 265 1951
10 Morrow G Methylmalonic acidemia *In* W L Nyhan (ed) *Heritable disorders of amino acid metabolism* John Wiley & Sons New York 1974
11 Nyhan W L Nonketotic hyperglycinemia *In* W L Nyhan (ed) *Heritable disorders of amino acid metabolism* John Wiley & Sons New York 1974
12 Perry T L Urquart N MacLean J Evans M E Hansen S Davidson A G F Applegarth D A MacLeod P J & Lock J E Nonketotic hyperglycinemia *N Engl J Med 292* 1269 1975
13 Sato T Kochi H Sato N & Kikuchi G Glycine metabolism by rat liver mitochondria *J Biochem 65* 77 1969
14 Snyder S H The glycine synaptic receptor in the mammalian central nervous system *Br J Pharmacol 53* 473 1975
15 Young A B & Snyder S H Strychnine binding associated with glycine receptors of the central nervous system *Proc Nat Acad Sci USA 70* 2832 1973

Submitted July 12 1978
Accepted Nov 25 1978

(S K) Institute of Human Genetics
The Bartholin Building
University of Aarhus
DK 8000 Aarhus C
Denmark

Acta Pædiatr Scand 68 635–638 1979

CASE REPORT

DELETION OF THE LONG ARM OF CHROMOSOME 11

A Clinical Entity

I FELDING and F MITELMAN

From the Department of Paediatrics Central Hospital Helsingborg and Department of Clinical Genetics Lund University Hospital Lund Sweden

ABSTRACT Felding I and Mitelman F (Department of Paediatrics Central Hospital Helsingborg and Department of Clinical Genetics Lund University Hospital Lund Sweden) Deletion of the long arm of chromosome No 11 A clinical entity Acta Paediatr Scand 68 635 1979 —A deletion of the long arm of chromosome No 11 an aberration undetectable in conventional chromosome staining was identified with Giemsa banding in a female infant with multiple congenital anomalies A survey is given of the clinical findings in the few cases so far reported

KEY WORDS Chromosome aberration banding technique congenital anomalies

The introduction of chromosome banding techniques has been of great importance for the delineation of new clinical syndromes The purpose of this paper is to describe the clinical findings in patients with a structural chromosome aberration which has escaped detection with the conventional staining technique



Fig 1 Appearance of the newborn baby



Fig 2 Appearance of the patient at the age of 4 months

that the treatment was initiated when he was 26 months old and already severely brain damaged

ACKNOWLEDGEMENT

This work was supported by the Danish Medical Research Council

REFERENCES

1 Ando T & Nyhan W L Propionic acidemia and the ketotic hyperglycinemia syndrome *In* W L Nyhan (ed) *Heritable disorders of amino acid metabolism* John Wiley & Sons New York 1974
2 Brandt N J Rasmussen K Brandt S Kolvraa S & Schonheyder F D-glycerie acidemia and nonketotic hyperglycinemia *Acta Paediatr Scand* *65* 17 1976
3 Childs B Nyhan W I Borden M Bard L & Cook R E Idiopathic hyperglycinemia and hyperglycinuria A new disorder of amino acid metabolism *Pediatrics* 27 522 1961
4 Gitzelmann R Cuenod M Otten A Steinmann B & Dumermuth G Nonketotic hyperglycinemia treated with strychnine *Pediatr Res* *11* 1016 1977
5 Hillman R E & Keating J P β ketothiolase deficiency as a cause of the ketotic hyperglycinemia syndrome *Pediatrics* *53* 221 1974
6 Krieger J Winbaum E S & Eisenbrey A B Cerebro-spinal fluid glycine in nonketotic hyperglycinemia Effect of treatment with sodium benzoate and a ventricular shunt *Metabolism* 26 517 1977
7 Kolvraa S Rasmussen K & Brandt N J D-glyceric acidemia biochemical studies of a new syndrome *Pediatr Res* *10* 825 1976
8 Lajtha A Amino acid transport in the brain in vivo and in vitro *In* G E W Wolstenholm & D W Fitzsimmons (eds) *Aromatic amino acids in the brain* Ciba Foundation Symp 22 p 25 Elsevier New York 1974
9 Lowry O H Rosebrough N J Farr A L & Randall R J *J Biol Chem* *193* 65 1951
10 Morrow G Methylmalonic acidemia *In* W L Nyhan (ed) *Heritable disorders of amino acid metabolism* John Wiley & Sons New York 1974
11 Nyhan W L Nonketotic hyperglycinemia *In* W L Nyhan (ed) *Heritable disorders of amino acid metabolism* John Wiley & Sons New York 1974
12 Perry T L Urquart N MacLean J Evans M E Hansen S Davidson A G F Applegarth D A MacLeod P J & Lock J E Nonketotic hyperglycinemia *N Engl J Med* *292* 1269 1975
13 Sato T Kochi H Sato N & Kikuchi G Glycine metabolism by rat liver mitochondria *Biochem* *65* 77 1969
14 Snyder S H The glycine synaptic receptor in mammalian central nervous system *Br J Pharmac* *53* 473 1975
15 Young A B & Snyder S H Strychnine binding associated with glycine receptors of the central nervous system *Proc Nat Acad Sci USA* *70* [illegible] 1973

Submitted July 12 1978
Accepted Nov 25 1978

(S K) Institute of Human Genetics
The Bartholin Building
University of Aarhus
DK 8000 Aarhus C
Denmark

Small for gestational age	●	●	●	●	●	●							
Post natal growth retardation	●	●	(●)	●	●	●	●	●	●	●	●		
Mental and motor retardation	●	●	(●)	●	●	●	●	●	●	●	●	(●)	●
Hand and foot deformities	●	●	●	●	●	●	●	●	●	●	●	●	
Abnormal skull shape (trigonocephaly)	●	●	●	●	●	●	●	●	●	●	●		
Broad bridged nose	●	●	●	●	●	●	●	●	●	●	●		
Micrognathia	●	●	●	●	●	●	●	●	●	●	●		
Carp-shaped mouth	●	●	●	●	●	●	●	●	●	●			
Ear abnormalities	●	●	●	●	●	●	●	●	●	●			
Congenital heart disease	●	●	●	●	●	●	●	●	●				
Frequent infections	●	●	(●)	●	●	●	●	●	(●)				
Hypertelorism	●	●	●	●	●	●	●	●	●	●			
Eye abnormalities	●	●	●	●	●	●	●						
High arched palate	●	●	●	●	●	●							
Simian creases	●	●	●	●	●	●	●	●					

Fig 4 Major clinical symptoms in reported cases of 46 XX del(11) (q 1-q?4) 13 ♀ 2♂

Routine urinary metabolic screening including aminoacid urinary excretion normal Cerebrospinal fluid normal Lysosomal enzymes including β galactosidase β glucosidase α mannosidase α fucosidase and β hexosaminidase normal Electrocardiogram pattern of ventricular strain Electroencephalogram normal for age

Radi g aphic examination

Thin skull but otherwise normal bones Normal hands and feet and corresponding to age except for an ossified phalanx in the sixth toe and supernumerary fingers without ossification Pelvis and hip-joints normal Chest X ray showed cardiac enlargement and dilated lung vessels Oesophagus and ventricle normal

DISCUSSION

So far 14 cases with a terminal deletion of the long arm of chromosome No 11 have been reported by Coco & Penchaszadeh (1) Engel et al (2) Faust et al (3) Frank & Riccardi (4) Jacobsen et al (5) Kaffe et al (6) Larson et al (7) Linarelli et al (8) Mulichy & Jenkyn (9) Turleau et al (10) and Zabel et al (11)

It might be of interest that females have accounted for 13 of the 15 cases with this deletion Only in one family was the deletion inherited through a translocation carrier parent (5) With the exception of one case (3) showing a more unspecific dysplastic appearance though loss of apparently the same amount of genetic material all other cases including our patient had the following main characteristics with varying phenotypic expressions intrauterine growth failure deficient height and weight gain motor and mental retardation frequent infections abnormal skull with trigonocephaly flat broad nasal bridge high arched palate micrognathia carp mouth lowset abnormal ears eye abnormalities clenched hands with simian creases foot malformations and congenital heart disease

REFERENCES

1 Coco R & Penchaszadeh V B Partial deficiency of long arm of chromosome No 11 *J Ge iet Hum* 25 43 1977

2 Engel E Heirschberg C S Cassidy S B & McGee B J Chromosome 11 long arm partial deletion A new syndrom *Am J Ment Defic* 80 473 1976

3 Faust J Vogel W & Loning B A case with 46 XX del (11) (q?1) *Clin Genet* 6 90 1974

4 Frank J & Riccardi V M The 11q syndrom *Hum Genet* 35 41 1977

5 Jacobsen P Hauge M Henningsen K Hobolth N Mikkelsen M & Philip J An (11 ?1) translocation in four generations with chromosome 11 abnormalities in the offspring *Hum Hered* ?3 568 1973

6 Kaffe S Hsu L Sachdev R Philips J & Hirschhorn K Partial deletion of long arm of chromosome 11 del (11) (q 3) *Clin Genet* 1? 3?3 1977

7 Larson S A Yeatman S W & Riccardi V M Deletion of 11q Report of two cases and a review *Birth Defects Original Article Series* vol XII 1 5 1976

8 Linarelli L G Gopalkrishna Pai K Pan S F & Rubin H M Anomalies associated with partial

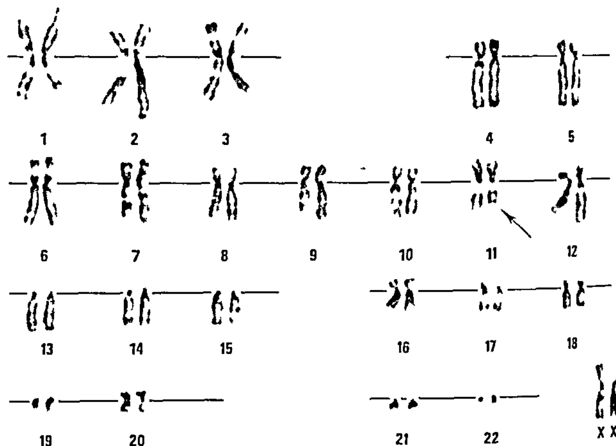


Fig 3 Trypsin Giemsa banded karyotype showing a terminal deletion (arrow) of chromosome 11 del(11) (q22)

CASE REPORT

Clinical findings

Our patient a girl born in 1975 was the second child of healthy unrelated parents both 30 years old The elder brother is healthy There was no history of abortions no family history of congenital defects and no exposure of parents or fetus to medical treatment infection or radiation After an uneventful pregnancy spontaneous delivery occurred at 36 weeks gestation Birth weight 1580 g length 46 cm small for gestational age Apgar score at 1 and 5 min 8

The placenta weighed 375 g and showed regressive changes oedema and hemorrhage some villi were hyalinized There was no evidence of intrauterine infection measured by total IgM not even after special separation for rubella and cytomegalvirus infection Virus isolation of samples from mother and infant were negative as well as serologic tests for toxoplasmosis rubella cytomegal virus and herpes simplex

The infant had a distinctive appearance trigonocephaly with thin soft bones and large sutures low set slightly malformed ears elongated philtrum carp shaped mouth micrognathia nose depressed at the base hypertelorism high arched palate colobomes of the eyelids but no other eye malformation only progressive pigmentation of the fundus clenched hands with simian creases supernumerary fingers on both hands and a sixth toe on the right foot A cardiac systolic murmur was heard compatible with a ventricular septal defect

The initial floppiness was followed by a generally increased tonus The motor and mental development was retarded after some months almost unprogressive Our patient showed a generally reduced vitality with continuous failure to thrive the condition dominated by cardiac incompensation and feeding difficulties She had recurrent urinary and upper respiratory tract infection After some accidents of aspiration she died of pneumonia at the age of 6½ months

Cytogenetic findings

Chromosome analysis of peripheral lymphocytes shortly after birth showed a normal female karyotype (46 XX) using conventional Giemsa staining Repeated studies on leukocyte and skin fibroblast cultures by means of a trypsin Giemsa banding technique however revealed a consistent structural abnormality a deletion of part of the long arm of one chromosome No 11 the breakpoint located at band q22 Both parents had normal karyotypes and prenatal diagnosis performed in a subsequent pregnancy also demonstrated a normal karyotype

Laboratory investigations

Blood counts normal except for a transient thrombocytopenia No vacuolated lymphocytes Blood glucose electrolytes thyroid liver and renal function tests normal

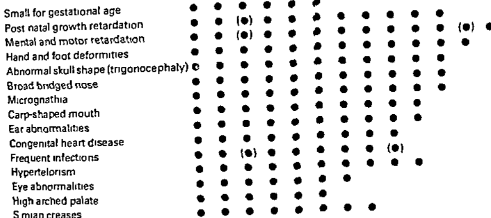


Fig 4 Major clinical symptoms in reported cases of 46 XX del(11) (q?1–q?4) 13 ♀ ?♂

Routine urinary metabolic screening including aminoacid urinary excretion normal Cerebrospinal fluid normal Lysosomal enzymes including β galactosidase β glucosidase α mannosidase α fucosidase and β hexosaminidase normal Electrocardiogram pattern of ventricular strain Electroencephalogram normal for age

Radiographic examination

Thin skull but otherwise normal bones Normal hands and feet and corresponding to age except for an ossified phalanx in the sixth toe and supernumerary fingers without ossification Pelvis and hip-joints normal Chest X ray showed cardiac enlargement and dilated lung vessel Oesophagus and ventricle normal

DISCUSSION

So far 14 cases with a terminal deletion of the long arm of chromosome No 11 have been reported by Coco & Penchaszadeh (1) Engel et al (2) Faust et al (3) Frank & Riccardi (4) Jacobsen et al (5) Kaffe et al (6) Larson et al (7) Linarelli et al (8) Mulachy & Jenkyn (9) Turleau et al (10) and Zabel et al (11)

It might be of interest that females have accounted for 13 of the 15 cases with this deletion Only in one family was the deletion inherited through a translocation carrier parent (5) With the exception of one case (3) showing a more unspecific dysplastic appearance though loss of apparently the same amount of genetic material all other cases including our patient had the following main characteristics with varying phenotypic expressions intrauterine growth failure deficient height and weight gain motor and mental retardation frequent infections abnormal skull with trigonocephaly flat broad nasal bridge high arched palate micrognathia carp mouth lowset abnormal ears eye abnormalities clenched hands with simian creases foot malformations and congenital heart disease

REFERENCES

1 Coco R & Penchaszadeh V B Partial deficiency of long arm of chromosome No 11 *J Genet Hum* ?5 43 1977

? Engel E Hirschberg C S Cassidy S B & McGee B J Chromosome 11 long arm partial deletion A new syndrom *Am J Ment Defic 80* 473 1976

3 Faust J Vogel W & Loning B A case with 46 XX del (11) (q?1) *Clin Genet* 6 90 1974

4 Frank J & Riccardi V M The 11q syndrom *Hum Genet 35* 241 1977

5 Jacobsen P Hauge M Henningsen K Hobolth N Mikkelsen M & Philip J An (11 ;21) translocation in four generations with chromosome 11 abnormalities in the offspring *Hum Hered 23* 568 1973

6 Kaffe S Hsu L Sachdev R Philips J & Hirschhorn K Partial deletion of long arm of chromosome 11 del (11) (q?3) *Clin Genet 12* 3?3 1977

7 Larson S A Yeatman S W & Riccardi V M Deletion of 11q Report of two cases and a review *Birth Defects Original Article Series* vol XII 1 5 1976

8 Linarelli L G Gopalkrishna Pai K Pan S F & Rubin H M Anomalies associated with partial

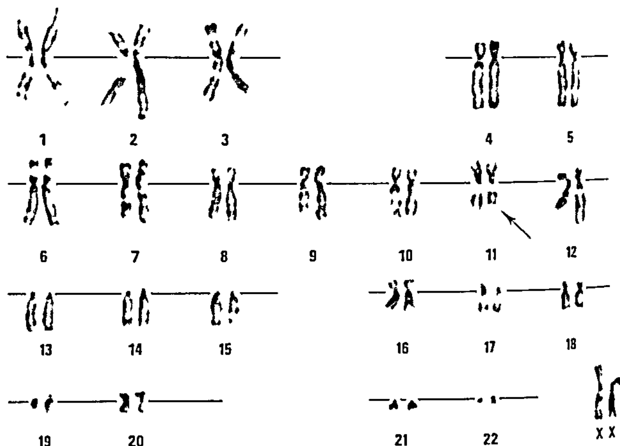


Fig. 3. Trypsin Giemsa banded karyotype showing a terminal deletion (arrow) of chromosome 11 del(11) (q22).

CASE REPORT

Clinical findings

Our patient, a girl born in 1975, was the second child of healthy unrelated parents, both 30 years old. The elder brother is healthy. There was no history of abortions, no family history of congenital defects and no exposure of parents or fetus to medical treatment, infection or radiation. After an uneventful pregnancy spontaneous delivery occurred at 36 weeks gestation. Birth weight 1 580 g, length 46 cm, small for gestational age. Apgar score at 1 and 5 min: 8.

The placenta weighed 375 g and showed regressive changes: oedema and hemorrhage; some villi were hyalinized. There was no evidence of intrauterine infection measured by total IgM, not even after special separation for rubella and cytomegalovirus infection. Virus isolation of samples from mother and infant were negative as well as serologic tests for toxoplasmosis, rubella, cytomegalovirus and herpes simplex.

The infant had a distinctive appearance: trigonocephaly with thin, soft bones and large sutures, low set, slightly malformed ears, elongated philtrum, carp shaped mouth, micrognathia, nose depressed at the base, hypertelorism, high arched palate, colobomes of the eyelids but no other eye malformation, only progressive pigmentation of the fundus, clenched hands with simian creases, supernumerary fingers on both hands and a sixth toe on the right foot. A cardiac systolic murmur was heard compatible with a ventricular septal defect.

The initial floppiness was followed by a generally increased tonus. The motor and mental development was retarded, after some months almost unprogressive. Our patient showed a generally reduced vitality with continuous failure to thrive, the condition dominated by cardiac incompensation and feeding difficulties. She had recurrent urinary and upper respiratory tract infections. After some accidents of aspiration she died of pneumonia at the age of 6½ months.

Cytogenetic findings

Chromosome analysis of peripheral lymphocytes shortly after birth showed a normal female karyotype (46, XX) using conventional Giemsa staining. Repeated studies on leukocyte and skin fibroblast cultures by means of a trypsin Giemsa banding technique, however, revealed a consistent structural abnormality: a deletion of part of the long arm of one chromosome No. 11, the breakpoint located at band q22. Both parents had normal karyotypes and prenatal diagnosis, performed in a subsequent pregnancy, also demonstrated a normal karyotype.

Laboratory investigations

Blood counts normal, except for a transient thrombocytopenia. No vacuolated lymphocytes. Blood glucose, electrolytes, thyroid, liver and renal function tests normal.

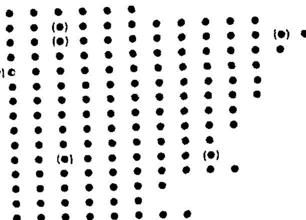


Fig 4 Major clinical symptoms in reported cases of 46,XX del(11) (q?1–q 4) 13 ♀ 2♂

Routine urinary metabolic screening including aminoacid urinary excretion normal Cerebrospinal fluid normal Lysosomal enzymes including β galactosidase β glucosidase α mannosidase α fucosidase and β hexosaminidase normal Electrocardiogram pattern of ventricular strain Electroencephalogram normal for age

Radiographic examination

Thin skull but otherwise normal bones Normal hands and feet and corresponding to age except for an ossified phalanx in the sixth toe and supernumerary fingers without ossification Pelvis and hip-joints normal Chest X ray showed cardiac enlargement and dilated lung vessel Oesophagus and ventricle normal

DISCUSSION

So far 14 cases with a terminal deletion of the long arm of chromosome No 11 have been reported by Coco & Penchaszadeh (1) Engel et al (2) Faust et al (3) Frank & Riccardi (4) Jacobsen et al (5) Kaffe et al (6) Larson et al (7) Linarelli et al (8) Mulachy & Jenkyn (9) Turleau et al (10) and Zabel et al (11)

It might be of interest that females have accounted for 13 of the 15 cases with this deletion Only in one family was the deletion inherited through a translocation carrier parent (5) With the exception of one case (3) showing a more unspecific dysplastic appearance though loss of apparently the same amount of genetic material all other cases including our patient had the following main characteristics with varying phenotypic expressions intrauterine growth failure deficient height and weight gain motor and mental retardation frequent infections abnormal skull with trigonocephaly flat broad nasal bridge high arched palate micrognathia carp mouth lowset abnormal ears eye abnormalities clenched hands with simian creases foot malformations and congenital heart disease

REFERENCES

1 Coco R & Penchaszadeh V B Partial deficiency of long arm of chromosome No 11 *J Genet Hum 25* 43 1977
2 Engel E Heirschberg C S Cassidy S B & McGee B J Chromosome 11 long arm partial deletion A new syndrom *Am J Ment Defic 80* 473 1976
3 Faust J Vogel W & Loning B A case with 46 XX del(11)(q?1) *Clin Genet* 6 90 1974
4 Frank J & Riccardi V M The 11q syndrom *Hum Genet 35* 241 1977
5 Jacobsen P Hauge M Henningsen K Hobolth N Mikkelsen M & Philip J An (11 21) translocation in four generations with chromosome 11 abnormalities in the offspring *Hum Hered* 23 568 1973
6 Kaffe S Hsu L Sachdev R Philips J & Hirschhorn K Partial deletion of long arm of chromosome 11 del(11)(q?3) *Clin Genet 12* 3?3 1977
7 Larson S A Yeatman S W & Riccardi V M Deletion of 11q Report of two cases and a review *Birth Defects Original Article Series* vol XII 1 5 1976
8 Linarelli L G Gopalkrishna Pai K Pan S F & Rubin H M Anomalies associated with partial

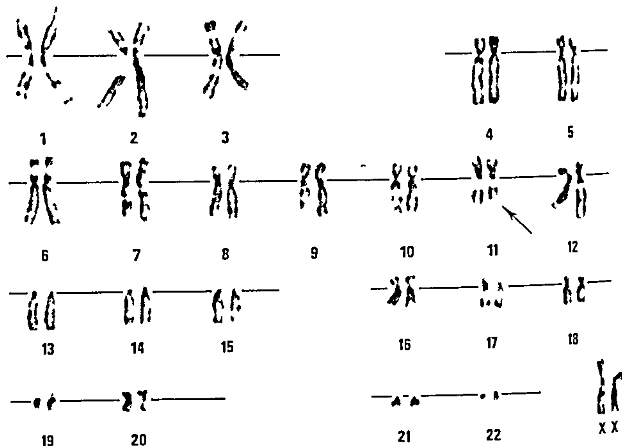


Fig 3 Trypsin Giemsa banded karyotype showing a terminal deletion (arrow) of chromosome 11 del(11) (q22)

CASE REPORT

Clinical findings

Our patient a girl born in 1975 was the second child of healthy unrelated parents both 30 years old The elder brother is healthy There was no history of abortions no family history of congenital defects and no exposure of parents or fetus to medical treatment infection or radiation After an uneventful pregnancy spontaneous delivery occurred at 36 weeks gestation Birth weight 1580 g length 46 cm small for gestational age Apgar score at 1 and 5 min 8

The placenta weighed 375 g and showed regressive changes oedema and hemorrhage some villi were hyalinized There was no evidence of intrauterine infection measured by total IgM not even after special separation for rubella and cytomegalvirus infection Virus isolation of samples from mother and infant were negative as well as serologic tests for toxoplasmosis rubella cytomegal virus and herpes simplex

The infant had a distinctive appearance trigonocephaly with thin soft bones and large sutures low set slightly malformed ears elongated philtrum carp shaped mouth micrognathia nose depressed at the base hypertelorism high arched palate colobomes of the eyelids but no other eye malformation only progressive pigmentation of the fundus clenched hands with simian creases supernumerary fingers on both hands and a sixth toe on the right foot A cardiac systolic murmur was heard compatible with a ventricular septal defect

The initial floppiness was followed by a generally increased tonus The motor and mental development was retarded after some months almost unprogressive Our patient showed a generally reduced vitality with continuous failure to thrive the condition dominated by cardiac incompensation and feeding difficulties She had recurrent urinary and upper respiratory tract infections After some accidents of aspiration she died of pneumonia at the age of 6½ months

Cytogenetic findings

Chromosome analysis of peripheral lymphocytes shortly after birth showed a normal female karyotype (46 XX) using conventional Giemsa staining Repeated studies on leukocyte and skin fibroblast cultures by means of a trypsin Giemsa banding technique however revealed a consistent structural abnormality a deletion of part of the long arm of one chromosome No 11 the breakpoint located at band q22 Both parents had normal karyotypes and prenatal diagnosis performed in a subsequent pregnancy also demonstrated a normal karyotype

Laboratory investigations

Blood counts normal except for a transient thrombocytopenia No vacuolated lymphocytes Blood glucose electrolytes thyroid liver and renal function tests normal

Acta Pædiatr Scand 68 639–641 1979

REVIEW OF CURRENT LITERATURE

THE LITERARY JUNGLE OF CYSTIC FIBROSIS

During the last few years the literature concerning cystic fibrosis (CF) has been overwhelming A search by Medlars for CF has yielded about twenty articles per month and added to these are review articles proceedings from international meetings manuals academic theses and so on This brief and incomplete review of the publications on CF is intended as a guide to help readers to find those works which are of most value for their special interest

Three review articles (1–3) were published in 1976 In the New England Journal of Medicine Paul di Sant Agnese & Pamela Davis (1) give a survey of the research in CF which is a direct continuation of an earlier survey in the same journal (1967) The survey is extensive and covers nearly all essential research on the pathogenesis of CF The findings are reported in an objective way and the authors are careful not to put forward hypotheses of their own They conclude however that CF must account for a generalized exocrinopathy but with morphologic and physiologic normality of the exocrine glands before the onset of the pathologic effects of disease Control of the secretory process is a possible site of the defect The review does not deal with clinical research on CF and gives no suggestions for the treatment and clinical care of patients with the disease

The review by Robert E Wood et al (2) comprising 46 pages with 503 references covers all aspects of CF—the incidence genetics pathophysiology clinical and pathologic manifestations diagnosis treatment complications psychosocial aspects and prognosis The authors have thorough knowledge of both the research and clinical management of CF and their description of the state of the art in CF is a result of their own experience combined with reports from the literature The Cleveland theories regarding both the pathophysiology and treatment of CF are easily recognized This is no drawback The results obtained by this group both in their research and in their clinical work have been excellent and in many aspects have led the views on CF ever since the days of Leroy W Matthews Comprehensive and intensive therapy is mandatory in CF The balanced discussion about mist tent therapy inhalations antibiotic treatment physical therapy diet and other therapeutic aids should be read by everyone concerned with the care of CF patients

The third review by Bowman & Barnett (3) deals mainly with those parts of CF research in which the Texas group has been most interested namely CF factor(s) and the possibilities of prenatal diagnosis Their review sums up the articles in the special CF issue of Texas Reports on Biology and Medicine (6)

The book of proceedings from the international conference Cystic Fibrosis—projections into the future (4) held in Israel in May 1976 is somewhat uneven but gives some exciting views on what can be done in CF research Hopefulness and constructive ideas for future directions predominate even though the description of CF research given by Benke may easily give rise to despair

There is the suggestion that cell membrane surfaces are abnormal and normal beta glucuronidase activity is abnormal and normal fatty acids are abnormal and normal serum calcium binding is abnormal and normal

deletion of long arm of chromosome 11 J Paediatr 86 750 1975
9 Mulchy M T & Jenkyn J The 11q syndrom Another case report Hum Genet 36 239 1977
10 Turleau C Chavin-Collin F Roubin M Thomas D & deGrouchy J Monosomie partielle 11q et trigonocephalie Un nouveau syndrome Ann Genet 18 257 1975
11 Zabel B Hansen S Hillig U & Grotig Imhof H A girl with partial long arm deletion of chromosome 11 and familial pericentric inversion of chromosome 9 Hum Genet 36 117 1977

Submitted Aug 25 1978
Accepted Dec 21 1978

(I F) Department of Paediatrics
Central Hospital
S 251 87 Helsingborg
Sweden

? Wood R E Boat T F & Doershuk C F Cystic fibrosis *Am Re\ Resp Dis 113* 833–878 1976
3 Bowman B H & Barnett D R Recent advances in cystic fibrosis research *Birth defects Original article series* XII 6 197–?1 1976

Proceedings etc

4 Mangos J A & Talamo R C (eds) *C\stic fib osis Projections int\ the future* Stratton Intercontinental Medical Book Corporation Miami Florida 1976
5 *Proceedings \IIth International C\stic Fibrosis Con g ess Pa is 1976* Imprimerie Jouve Paris 1978
6 Bowman B H & Barnett D R (eds) Cystic fibrosis *Texas Reports on Biolog\ and \ledicine 34* 1 ?19 (=whole no 1) 1976
7 Forstner G G (ed) *\lucus secretions and c\stic fibrosis* S Karger Basel 1977
8 Baran D & van Bogaert E (eds) *Chest ph\sical therap\ in c\stic fibrosis and chronic obstructi\e pulmonar\ diseases* European Press Gent 1977

\lanuals

9 Anderson C M & Goodchild M C *C\stic fibrosis \lanual of diagnosis and management* Blackwell Oxford 1976
10 Bender S W & Stephan U *\lucoviscidose C\stische Fibrose* Deutsche Gesellschaft zur Bekampfung der Mucoviscidose Erlangen 1977
11 Doershuk C F (ed) *Guide to diagnosis and management of c\stic fibrosis* National Cystic Fibrosis Research Foundation Atlanta USA 1971
1? Huang N N (ed) *Guide to d ug therap\ in patients \ ith cystic fibrosis* National Cystic Fibrosis Research Foundation Atlanta 197
13 Kjellmer I Kollberg H Kornfalt R Lindberg T & Strandvik B Behandling av cystisk fibros *Lakartidningen 10* 855 1976

polyamines in blood are high and normal RNA methylation is low and normal calcium or magnesium ATPase and sodium transport are abnormal and normal active sodium transport is affected and not affected by CF materials complement levels are elevated and normal the Tamm Horsfall glycoprotein is aggregated and normal glycosyl transferases are low high and normal the cell cycle is abnormal and normal arginine is important and not important kallikrein is abnormal and normal a CF factor can be demonstrated and not demonstrated glutathione reductase is high in red cells and low in white cells arginine esterase is abnormal and normal and CF cells have excess metachromatic material but this is normal and besides CF cells are sick and functional failures The clinical part of the book deals with pulmonary cardiovascular gastrointestinal and nutritional features of the disease mostly focusing on a few special aspects centered mainly on the authors own investigations It is not intended for providing clinicians with straight guide lines for treatment and they may be disappointed on this account The second part on fundamental problems of biochemistry immunology cell and molecular biology CF factors and genetics is much more intriguing Cells of CF affected individuals seem to be triggered into a stimulatory loop by a hitherto unknown factor Articles by Farell & Lundgren Butcher and Lederberg do much to link the many contradictory findings together The book is valuable for those who want to know where the frontier of CF research lay in 1976 and who want to get ideas about how to go on further

The other reports of proceedings (5–8) are good complements to the Projections into the future The proceedings of the Paris meeting (5) cover the whole field from basic to clinical research Each of the others (6–8) is concentrated on a special problem and will therefore be of most value for those interested in the field in question The Texas report (6) focuses on cell cultures and CF factors the Canadian report (7) on mucus secretions and mucociliary transport and the Belgian report (8) on chronic obstructive pulmonary disease and physical therapy

It is not possible to make a critical review of each of the contributions in the proceedings The books are essential for those who are interested in CF and are working on research projects in this or adjacent fields However there are a few points that must be underlined CF patients should in general be more strictly defined (5–8) The diagnosis of CF is difficult and only patients with elevated sweat chlorides and at least one of the major clinical symptoms from the lungs pancreas or meconium ileus should be included in this category Some of the divergences might be due to improper selection of patients Also the reference material should be well documented (5–8) E g To use material from patients with different kinds of inborn errors of metabolism as control material for cAMP concentrations in fibroblasts is unacceptable (7) Culture conditions (5–7) should be described in detail including the type of medium the routine for exchange of medium the type and concentration of serum and the number of passages Differences in all these variables have been proven to alter the behaviour of CF cells (6)

The two manuals (9–10) give no new ideas for treatment of CF The article by Wood et al (2) and the last issues of the Guides to Cystic Fibrosis (11–12) give all the necessary information in a more balanced and objective manner For Scandinavian readers the report on the recent symposium in Lakartidningen (13) might also be used as a manual for the diagnosis and management

Hans Kollberg

Department of Paediatrics
University Hospital
901 85 Umeå
Sweden

Review articles

1 di Sant Agnese P A & Davis P B Research in cystic fibrosis *N Engl J Med* 295 481–485 534–541 and 597–602 1976

2 Wood R E Boat T F & Doershuk C F Cystic fibrosis *Am Rev Resp Dis 113* 833–878 1976
3 Bowman B H & Barnett D R Recent advances in cystic fibrosis research *Birth defects Original article series* XII 6 197–212 1976

Proceedings etc

4 Mangos J A & Talamo R C (eds) *Cystic fibrosis Projections into the future* Stratton Intercontinental Medical Book Corporation Miami Florida 1976
5 *Proceedings VIIth International Cystic Fibrosis Congress Paris 1976* Imprimerie Jouve Paris 1978
6 Bowman B H & Barnett D R (eds) Cystic fibrosis *Texas Reports on Biology and Medicine 34* 1 19 (=whole no 1) 1976
7 Forstner G G (ed) *Mucus secretions and cystic fibrosis* S Karger Basel 1977
8 Baran D & van Bogaert E (eds) *Chest physical therapy in cystic fibrosis and chronic obstructive pulmonary diseases* European Press Gent 1977

Manuals

9 Anderson C M & Goodchild M C *Cystic fibrosis Manual of diagnosis and management* Blackwell Oxford 1976
10 Bender S W & Stephan U *Mucoviscidose Cystische Fibrose* Deutsche Gesellschaft zur Bekampfung der Mucoviscidose Erlangen 1977
11 Doershuk C F (ed) *Guide to diagnosis and management of cystic fibrosis* National Cystic Fibrosis Research Foundation Atlanta USA 1971
12 Huang N N (ed) *Guide to drug therapy in patients with cystic fibrosis* National Cystic Fibrosis Research Foundation Atlanta 1972
13 Kjellmer I Kollberg H Kornfalt R Lindberg T & Strandvik B Behandling av cystisk fibros *Lakartidningen 10* 855 1976

polyamines in blood are high and normal RNA methylation is low and normal calcium or magnesium ATPase and sodium transport are abnormal and normal, active sodium transport is affected and not affected by CF materials complement levels are elevated and normal the Tamm Horsfall glycoprotein is aggregated and normal glycosyl transferases are low high and normal the cell cycle is abnormal and normal arginine is important and not important kallikrein is abnormal and normal a CF factor can be demonstrated and not demonstrated glutathione reductase is high in red cells and low in white cells arginine esterase is abnormal and normal and CF cells have excess metachromatic material but this is normal and besides CF cells are sick and functional failures The clinical part of the book deals with pulmonary cardiovascular gastrointestinal and nutritional features of the disease mostly focusing on a few special aspects centered mainly on the authors own investigations It is not intended for providing clinicians with straight guide lines for treatment and they may be disappointed on this account The second part on fundamental problems of biochemistry immunology cell and molecular biology CF factors and genetics is much more intriguing Cells of CF affected individuals seem to be triggered into a stimulatory loop by a hitherto unknown factor Articles by Farell & Lundgren Butcher and Lederberg do much to link the many contradictory findings together The book is valuable for those who want to know where the frontier of CF research lay in 1976 and who want to get ideas about how to go on further

The other reports of proceedings (5–8) are good complements to the Projections into the future The proceedings of the Paris meeting (5) cover the whole field from basic to clinical research Each of the others (6–8) is concentrated on a special problem and will therefore be of most value for those interested in the field in question The Texas report (6) focuses on cell cultures and CF factors the Canadian report (7) on mucus secretions and mucociliary transport and the Belgian report (8) on chronic obstructive pulmonary disease and physical therapy

It is not possible to make a critical review of each of the contributions in the proceedings The books are essential for those who are interested in CF and are working on research projects in this or adjacent fields However there are a few points that must be underlined CF patients should in general be more strictly defined (5–8) The diagnosis of CF is difficult and only patients with elevated sweat chlorides and at least one of the major clinical symptoms from the lungs pancreas or meconium ileus should be included in this category Some of the divergences might be due to improper selection of patients Also the reference material should be well documented (5–8) E g To use material from patients with different kinds of inborn errors of metabolism as control material for cAMP concentrations in fibroblasts is unacceptable (7) Culture conditions (5–7) should be described in detail including the type of medium the routine for exchange of medium the type and concentration of serum and the number of passages Differences in all these variables have been proven to alter the behaviour of CF cells (6)

The two manuals (9–10) give no new ideas for treatment of CF The article by Wood et al (2) and the last issues of the Guides to Cystic Fibrosis (11–12) give all the necessary information in a more balanced and objective manner For Scandinavian readers the report on the recent symposium in Lakartidningen (13) might also be used as a manual for the diagnosis and management

Hans Kollberg

Department of Paediatrics
University Hospital
901 85 Umeå
Sweden

Review articles

1 di Sant Agnese P A & Davis P B Research in cystic fibrosis N Engl J Med 295 481–485 534–541 and 597–602 1976

BOOK REVIEWS

D M Reed & F J Stanley *The epidemiology of prematurity* Urban & Schwarzenberg Munchen 1977 370 pp illus £18 50 ISBN 3 541 71611 8

This book provides a report from a working conference held at the National Institute of Child Health and Human Development Bethesda Maryland on Nov 8–9 1976 The conference tried to answer several questions concerning the risk of prematurity among different populations i e the epidemiology of prematurity With prematurity was meant various combinations of being born too early or too small or both

Most of the 35 contributors to the conference were from the United States a few from Great Britain and one from Norway The conference choose to go through a lot of previous work on prematurity e g reports from the British Perinatal Mortality Survey and from the Collaborative Perinatal Project in the U S It gives a good summary of factors in prematurity discussed for the last 10 to 20 years such as social and economical status race and parity

Cigarette smoking has been much in the limelight as an important factor in low birth weight and several papers are on this subject the most interesting perhaps by Mary B Meyar who compares cigarette smoking to living in a high altitude both conditions which deprive the fetus of oxygen Susser and Stein give an other interesting report comprising late results from the Dutch famine study and results from nutritional supplements in pregnancy—apparently the effects of nutrition on birth weight are not simple to evaluate Other papers deal with genetic aspects of prematurity and obstetric factors and prenatal care

The conclusions drawn from the conference were that prematurity is a diversiform condition which we must learn more about to be able to prevent The book is recommended to those who want a review of what is known today about the epidemiology of prematurity It gives however very few new facts or ideas to those who have followed the literature on this subject for the last ten years

Ingrid Bjer e

C G D Brook *Practical paediatric endocrinology* 15 pp illus Academic Press London 1978 £6 30 ISBN 0-12 136050-4

According to the author s preface the aim of this book is to help paediatricians with endocrinology and endocrinologists with paediatrics I think he has succeeded very well and presented a practical guide to the endocrine disorders of children and adolescents The book is not a conventional text book and is primarily written for those who have some basic knowledge of paediatric endocrinology but see children with endocrine diseases only occasionally It is however my impression that the book could also be used by the student as an introduction to this special field of paediatrics It is easily read and remarkably comprehensive The first part of each chapter gives a short and usually excellent physiological review which helps the reader to understand the abnormalities and the course of action to be taken It is possible as the author says in his preface that not all of his opinions are shared by other experts in this field but I think the advice given can always be followed without fear of any mistakes This practical book is highly recommended but for the next edition one would wish a better proof reading the present contains too many misprints

C G Bergstrand

J Borms & M Hebbelink (eds) *Paediatric work physiology* vol II *In* Medicine and Sport Series Eds E Jokl & H Hebbelink S Karger Basel 1978 178 pp illus D M 78 ISBN 3 8055 2866-3

Clinical physiological investigations are not only possible to perform in children—it is a positive surprise to anyone formerly only used to grown up patients to reveal how positive cooperative observant stimulating and kind most children are This is perhaps most evident in small patients marked by chronic diseases like bronchial asthma or cystic fibrosis Hopefully the results of clinical physiological investigations are rewarding to the child patient when providing better diagnoses and therapy Certainly the work with the child patient in itself is rewarding to the physician

This book which presents papers read at symposia at Seč Czechoslovakia in 1974 and at Bisham England in 1976 illustrates this thesis The introduction to work physiology in children and adolescents by E Jokl starts brilliantly but ends somewhat abruptly

The chapter on Physiological Adaptation presents methodology for work testing by threadmill or bicycle ergometer muscle blood flow measurements by 133 Xenon and effects of physical training on different body parameters

Under Clinical Pathology among other things there are found good reviews on bicycle exercise testing in cardiac patients and on the reaction to exercise in anorexia nervosa

Under Growth and Development are assembled eight papers on physical performance as related to age maturity and physical training The main advantage of this volume is that it gives a good idea about what can be measured in children presents different ways of doing so and provides good references for anybody interested in clinical physiology or sports medicine as applied to children Personally I enjoyed reading it

Hans Arborelius Jr

BOOK REVIEWS

D M Reed & F J Stanley *The epidemiology of prematurity* Urban & Schwarzenberg Munchen 1977 370 pp illus £18 50 ISBN 3 541 71611 8

This book provides a report from a working conference held at the National Institute of Child Health and Human Development Bethesda Maryland on Nov 8–9 1976 The conference tried to answer several questions concerning the risk of prematurity among different populations i e the epidemiology of prematurity With prematurity was meant various combinations of being born too early or too small or both

Most of the 35 contributors to the conference were from the United States a few from Great Britain and one from Norway The conference choose to go through a lot of previous work on prematurity e g reports from the British Perinatal Mortality Survey and from the Collaborative Perinatal Project in the U S It gives a good summary of factors in prematurity discussed for the last 10 to 0 years such as social and economical status race and parity

Cigarette smoking has been much in the limelight as an important factor in low birth weight and several papers are on this subject the most interesting perhaps by Mary B Meyar who compares cigarette smoking to living in a high altitude both conditions which deprive the fetus of oxygen Sus er and Stein give an other interesting report comprising late results from the Dutch famine study and results from nutritional supplements in pregnancy—apparently the effects of nutrition on birth weight are not simple to evaluate Other papers deal with genetic aspects of prematurity and obstetric factors and prenatal care

The conclusions drawn from the conference were that prematurity is a di ersiform condition which we must learn more about to be able to prevent The book is recommended to those who want a review of what is known today about the epidemiology of prematurity It gives however very few new facts or ideas to those who have followed the literature on this subject for the last ten years

Ingrid Bjerre

C G D Brook *P act cal paed atric endocrinol gy* 15? pp illus Academic Press London 1978 £6 30 ISBN 0-1 136050-4

According to the author s preface the aim of this book is to help paediatricians with endocrinology and endocrinologists with paediatrics I think he has succeeded very well and presented a practical guide to the endocrine disorders of children and adolescents The book is not a onventional text book and is primarily written for those who ha e some basic knowledge of paediatric endocrinol gy but see children with endocrine diseases only occa ionally It is however my impression that the book could also be used by the student as an introduction to this special field of paediatrics It is easily read and remarkably comprehensive The first part of each chapter gives a short and usually excellent physiological review which helps the reader to understand the abnormalities and the course of action to be taken It is possible as the author says in his preface that not all of his opinions are shared by other experts in this field but I think the advice given can always be followed without fear of any mistakes This practical book is highly recommended but for the next edition one would wish a better proof reading the present contains too many misprints

C G Bergstrand

J Borms & M Hebbelink (eds) *Paediatric work physiology* vol II *In* Medicine and Sport Series Eds E Jokl & H Hebbelink S Karger Basel 1978 178 pp illus D M 78 ISBN 3 8055 2866-3

Clinical physiological investigations are not only possible to perform in children—it is a positive surprise to anyone formerly only used to grown up patients to reveal how positive cooperative observant stimulating and kind most children are This is perhaps most evident in small patients marked by chronic diseases like bronchial asthma or cystic fibrosis Hopefully the results of clinical physiological investigations are rewarding to the child patient when providing better diagnoses and therapy Certainly the work with the child patient in itself is rewarding to the physician

This book which presents papers read at symposia at Seč Czechoslovakia in 1974 and at Bisham England in 1976 illustrates this thesis The introduction to work physiology in children and adolescents by E Jokl starts brilliantly but ends somewhat abrup ly

The chapter on Physiological Adaptation presents methodology for work testing by threadmill or bicycle ergometer muscle blood flow measurements by 133 Xenon and effects of physical training on different body parameters

Under Clinical Pathology among other things there are found good reviews on bicycle exercise testing in cardiac patients and on the reaction to exercise in anorexia nervosa

Under "Growth and Development are assembled eight papers on physical performance as related to age maturity and physical training The main advantage of this volume is that it gives a good idea about what can be measured in children presents different ways of doing so and provides good references for anybody interested in clinical physiology or sports medicine as applied to children Personally I enjoyed reading it

M ns Arborelius Jr

Acta Pædiatr Scand 68 645–648 1979

A CHILD S EXPERIENCE OF IMMINENT DEATH

INGELA NORDMARK LINDBERG and TOR LINDBERG

From the Department of Paediatrics Malmo General Hospital University of Lund Malmo Sweden

ABSTRACT Nordmark Lindberg I and Lindberg T (Department of Paediatrics Malmo General Hospital University of Lund Malmo Sweden) A child s experience of imminent death Acta Paediatr Scand 68 645 1979 —We relate here the experiences and thoughts of a 7 year-old girl about her situation during the last three months of life as she lay dying of Ewing s sarcoma Our experience with this girl—and that of other children in the same situation—indicates that they have a realistic and even confident view of death Their anxiety is centred on the disease and how it affects their appearance and their activity We most often underestimate children s capacity for understanding their own situation We ought to be sensitive to appeals for contact

KEY WORDS Children and death death dying

An intensive development is taking place in paediatrics as in other spheres of natural science and we are daily inundated with data concerning physiological and pathological happenings We run an obvious risk of being sidetracked and of forgetting the child—the person—behind the disease Paediatric literature contains very little about the sick child s experiences and how it regards its own situation and its possibly imminent death More information is essential

What is written about man s thoughts feelings and experiences of his own death and that of others is excellently summarized in Feigenberg s works (5 6) Very little deals with the child and death and then mainly with the thoughts and experiences of death by *healthy* children (1 7 8 9 11) Based on observations interviews and psychological tests those directly concerned have interpreted the thoughts of the dying child (3 4 10 12 15) Thus the adult s evaluation unconsciously influences the reports Several works have discussed the reactions of the family and staff and have proffered advice of how the dying child should be treated (1 3 4 12) Only few works (2 13 16) describe the child s spontaneous thoughts of its disease and imminent death

It is generally accepted that children s idea of death varies with their level of *development* and successively reaches that of the adult (1 11) We doubt whether this is valid at least for sick children The question is whether the actual situation—*the disease*—and the family s reaction to it is not of more decisive importance

Here we relate a 7 year-old girl s thoughts and feelings about her imminent death and how she experienced the reaction of those around her to her situation

CASE HISTORY

Eva was a first child with a ? year-old brother At 5 years of age she began to suffer with pains in the back She had a painful swelling over the lumbar region X ray disclosed skeletal destruction in the third lumbar vertebra The pathological anatomical diagnosis was Ewing s sarcoma She was treated at intervals with cytostatics and radiation Initially the effect was good with considerable freedom from symptoms for one year Thereafter there were signs of a slow decline with nonresponse of reflexes pains in back and legs and restricted movement The symptom increased After two years (at age 7) she became practically confined to a wheelchair Her liver increased in size and she had pain that demanded stronger

P G Ransley & R A Risdon *Reflux and renal scarring* British Journal of Radiology Suppl 14 1978 pp illus £3 60 (members of the British Institute of Radiology £3 00) ISBN 0306-8854

This work by Ransley & Risdon is an important contribution to elucidate the question whether renal scarring in vesicourethral reflux (VUR) can be caused by VUR alone or if concomitant urinary infection is an obligatory requirement In earlier now classical studies by Hodson it was found that high pressure sterile reflux chould be associated with renal scarring Moreover it was found that focal scar formation was related to intrarenal reflux (IRR) i e reflux of urine from calyx into renal parenchyma This point has been further investigated by Ransley & Risdon showing that susceptibility to IRR depends on the morphology of individual papillae Cone shaped papillae were found to be resistant to IRR because of a pressure mediated closure of the slit like orifices of the collecting ducts Compound coalescent papillae mostly found in the polar regions a common site for focal scars were because of their structure more easily transformed into refluxing papillae under the influence of high pressure

In a well controlled series of experiments in the growing pig the authors have now examined the question whether sterile IRR can cause renal scarring and the eventual role of pressure in scar formation The results of this study are unambiguous Under the experimental conditions used sterile reflux even under high pressure does not cause damage to the growing kidney Reflux in the presence of urinary infection will on the other hand readily produce renal scarring High pressure will intensify the renal scarring by damaging and transferring non refluxing papillae into refluxing papillae

The difference between these results and those earlier reported by Hodson is difficult to explain One explanation may be that Hodson s animals with sterile high pressure reflux could have been subjected to transient short lived infection which certainly will be able to cause scar formation It is reported in the present study that high pressure animals in spite of continuous chemotherapy are difficult to keep sterile because of the presence of residual urine Another explanation may be that the two studies were performed in different species of pigs

The applicability of these experimental findings to human VUR will have to await the results of further clinical studies Were they directly applicable it would seem illogical to reimplant ureters in children with VUR in order to prevent further renal damage In most cases surgery could then be replaced by adequate control of infection It is however premature to draw such conclusions The need for clinical follow up studies focused on the role of in tion for progression of functional deterioration is obv The authors are very cautious in the interpretation of results and refrain from any recommendations with re to surgery or non surgery in VUR Their work has a new and valuable information to our understandin reflux nephropathy It can be highly recommended reading to all those interested in paediatric urol ey

Ove Broberg

J B Hanshaw & J A Dudgeon *Viral diseases o fetus and newborn* (vol XVII in series Major Proble Clinical Pediatrics) W B Saunders Co Philadel London and Toronto 1978 347 pp illus £14 00 I 0-7216-4500-3

Viral infections represent an important problem in mo perinatology They may have short term as well as term effects upon fetal development and upon child birth Even if rather few viruses seem to be responsib different kinds of congenital defects they may be im tant in other respects The symptoms of disease w they may cause are extremely variable The same agent may cause a severe or even fatal disease in fetuses or newborns while in other cases there ar obvious signs of damage in spite of a proven infectio

This little volume is a most valuable contribution t series Major Problems in Clinical Pediatrics Th thors are wellknown for their important contributio our knowledge of cytomegalic infection and cong rubella respectively These infections are compre sively described in special chapters Other chapters an account of herpes simplex infections enterovi fections varicella zoster infections smallpox and cinia infections with hepatitis viruses and other pos pathogens in the fetus and newborn In one part o book the pathology of placenta and cord in some infections is described and in another part the dev ment of immune mechanisms in the fetus and new The book also contains chapters dealing with labor diagnosis and with prevention treatment and anti chemotherapy

In spite of the small size of the volume it is compre sive and gives a lot of actual theoreti al and prac information for the clinician The book is therefore hi recommended especially to those working in the peri field

Torsten Be

Acta Pædiatr Scand 68 645–648 1979

A CHILD S EXPERIENCE OF IMMINENT DEATH

INGELA NORDMARK LINDBERG and TOR LINDBERG

From the Department of Paediatrics Malmo General Hospital University of Lund Malmo Sweden

ABSTRACT Nordmark Lindberg I and Lindberg T (Department of Paediatrics Malmo General Hospital University of Lund Malmo Sweden) A child s experience of imminent death Acta Paediatr Scand 68 645 1979.—We relate here the experiences and thoughts of a 7 year-old girl about her situation during the last three months of life as she lay dying of Ewing s sarcoma Our experience with this girl—and that of other children in the same situation—indicates that they have a realistic and even confident view of death Their anxiety is centred on the disease and how it affects their appearance and their activity We most often underestimate children s capacity for understanding their own situation We ought to be sensitive to appeals for contact

KEY WORDS Children and death death dying

An intensive development is taking place in paediatrics as in other spheres of natural science and we are daily inundated with data concerning physiological and pathological happenings We run an obvious risk of being sidetracked and of forgetting the child—the person—behind the disease Paediatric literature contains very little about the sick child s experiences and how it regards its own situation and its possibly imminent death More information is essential

What is written about man s thoughts feelings and experiences of his own death and that of others is excellently summarized in Feigenberg s works (5 6) Very little deals with the child and death and then mainly with the thoughts and experiences of death by healthy children (1 7 8 9 11) Based on observations interviews and psychological tests those directly concerned have interpreted the thoughts of the dying child (3 4 10 12 15) Thus the adult s evaluation unconsciously influences the reports Several works have discussed the reactions of the family and staff and have proffered advice of how the dying child should be treated (1 3 4 12) Only few works (2 13 16) describe the child s spontaneous thoughts of its disease and imminent death

It is generally accepted that children s idea of death varies with their level of development and successively reaches that of the adult (1 11) We doubt whether this is valid at least for sick children The question is whether the actual situation—the disease—and the family s reaction to it is not of more decisive importance

Here we relate a 7 year old girl s thoughts and feelings about her imminent death and how she experienced the reaction of those around her to her situation

CASE HISTORY

Eva was a first child with a ? year-old brother At 5 years of age she began to suffer with pains in the back She had a painful swelling over the lumbar region X ray disclosed skeletal destruction in the third lumbar vertebra The pathological anatomical diagnosis was Ewing s sarcoma She was treated at intervals with cytostatics and radiation Initially the effect was good with considerable freedom from symptoms for one year Thereafter there were signs of a slow decline with nonresponse of reflexes pains in back and legs and restricted movement The symptoms increased After two years (at age 7) she became practically confined to a wheelchair Her liver increased in size and she had pain that demanded stronger

and stronger analgetics. At the age of 7 years and one month laminectomy and partial removal of extradural tumour tissue were carried out in unloading and pain reducing effort. After one month of relief the pain again increased, her abdomen swelled (liver enlargement and ascites) and a cough developed. She died at age 7 years and 4 months.

The family's (parents and maternal grandparents) relation with Eva was marked by an unusual frankness and lack of reserve, especially the relation between Eva and her mother. Eva's illness and imminent death were realistically discussed.

In the capacity of nursery school teacher, one of us (I. N. L.) achieved a close contact with Eva during her final three months of life. We relate here some of our conversations and experiences.

Eva's thoughts and experiences

Eva and I met for the first time a full three months before she died. She immediately made demands on me, and I responded with counterdemands. This was something she was not accustomed to. For a whole week we tested each other. Eva was then transferred to the neurosurgical department for operation. On the third day after the operation, she telephoned me at home to say "Good night". These "Good night" conversations continued until six days before she died. When Eva returned to the paediatric department, she definitely refused all forms of physiotherapy; no attempt was made to persuade her to change her mind. She immediately noticed this and became alarmed. She was angry and grumpy. I asked her if she wished to walk down to the play therapy. This aroused loud protests: 'Why are you so silly? you know that I can't walk." She did manage to walk as far as the door and then cried and swore. I took her into my arms, she laughed, cried and struggled a little. 'I can walk a bit, but some things I can't do." She could not explain the feeling. She said: "One feels so lonely, nobody understands." We often spoke of the war in Vietnam and the starvation in Ethiopia. She identified with their helplessness. "I am not the only one who can't manage things; the children in Ethiopia suffer a lot of pain and get nothing to eat. But when they die they will go to somewhere nice. If there is much pain before dying it must be such a relief to die, otherwise one would not die. When you die, you leave all those you like. But it is probably better to be dead because then you no longer suffer. But why do some people kill others? I am going to die because I am ill, and everyone is sorry, but when people are killed with guns in war, there is no sorrow. When I am in heaven, can I look down on you? God looks down on us now."

When Eva and I were on our way to Eva's home, we passed a cemetery. Eva said: "I will be buried here in a white coffin; all children have white coffins. Suppose my legs can't walk when I am in heaven, suppose the spider does not disappear, suppose ..." Someone who had visited her had said that one cannot be certain what will happen. "What if I go somewhere else?"

Her way of speaking was often provoking. She wished to test the truth of what we said and asked the same question again a few days later. It was important to give positive answers. If she failed to get a positive answer she fell silent and seemed confused. Sometimes she became very irritable with those around her. "Why do they say 'Little Eva, will you have this or do that?' and try to pat me on the cheek? I don't want to listen to their talk. They say things just because I am going to die."

Karin, 16 years of age, lay in the same ward. She had chronic and terminal myelocytic leukaemia. She had had treatment and was afraid that she would lose here hair. "I have also had the same treatment that Karin is having," Eva said. "You are given that when you have the illness we have. Does Karin know that she is going to die?" (Answer: "We all must die sometime.") "When you are given what Karin is having you will soon die." (Have you mentioned that to Karin?) "No. It would make her unhappy." (Won't you be unhappy?) Eva did not reply. "Karin does not know she is going

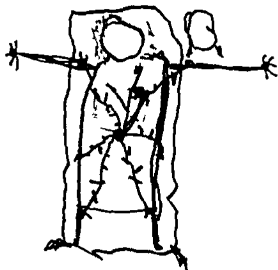


Fig 1 Eva s sketch of her tumour The spider s body is in the same place as the parent tumour There are tablets on the table (to the right)

to die because she talks about what she will do when she finishes school Eva said I feel sorry for her While we are at the shops Karin might die I heard the remark on the doctor s round that she has poor values

We noticed on our morning walks about three weeks before she died a beautiful dew spangled spider s web When Eva saw the spider she shuddered but was fascinated by its unpleasantness A few days later she said It feels as though I have a spider in my back He sits there and eats on my swelling The swelling is so large that the spider grows bigger and bigger He has long hairy legs I have an itch when he moves (Eva spoke and made a sketch at the same time Fig 1) He has a large foot that fastens in certain places Now he sits here (the lungs) which is why I am coughing I believe that there is one in my hand too because it shakes all the time Look how badly I draw now Sometimes I dream that the spider wraps his legs all round me and stops my breathing Don t tell anyone about the spider Mammy says that when anyone has the injections I get one has queer dreams but I know that my spider is real I know him He squeezes my throat all the time Can t you see how large he has become? He is fat because he eats me (points to her stomach) Soon he will put his foot into my heart and then I can t live any longer Then I will join the other children

A few days later she got a little doll as large as a finger with soft arms and legs She lay and looked at it with an expression of dis gust Look how ugly it is You can do any thing with its arms just as though it has no feelings She bent and twisted the doll s arms and legs in all manner of positions She prob ably has a spider all through her body Eva bit off the doll s legs bit by bit She shan t have any legs because I have none She shan t have arms either because I have none They shake all the time It is that ugly spider's fault Finally the doll s head came off She might just as well die you can t live without arms and legs Throw the nasty thing away

After that she wanted to be left in peace She did not want to talk nor to be left by her self Three days later she sank into coma which became increasingly deeper Three days later she passed peacefully into the beyond

DISCUSSION

This 7 year old girl s realistic concept of her illness and her imminent death agrees with both younger and older children s reactions which are—in more or less detail—recorded in the literature (2 13) These children s age related concept at death does not agree with that described concerning healthy children (1 11)

Eva s concept of life was influenced by the priest who was her Sunday teacher We did not feel that the thought of death held any terror for her but rather security This she also expressed in words and sketches she felt that God and his Angels would take care of her and that she would lie—as though in cot tonwool—in their arms On the other hand

she hated her illness and was filled with anxiety on account of it and how it affected her appearance and her mobility a reaction also described by others (3 10 15)

We are convinced that the case of Eva is by no means isolated but she was able to say what she thought Experience tells us that we adults rather underestimate the children s ability to understand their situation that we must be sensitive to their appeals (cf 14) Feigenberg (6) in his work about terminal care points to importance of one person being in close contact with the patient and becoming his confidant This applies also to children Moreover other personnel must be made aware of the phase the child is in Eva said that some parts of the conversations should not be repeated to her relatives and to her doctor in order not to make them feel sad

We have observed—not only through Eva—that children are sensitive to changes in the hospital routine changes that have been made because of deteriorations in the condition such as for instance the discontinuation of the physiotherapy for Eva Children also react when we cease to make demands on them Towards the end 16 year old Karin was allowed to be careless in her room She immediately noticed this and commented on it with some sarcasm

We must not fail to consider as far as possible the child s view of the practical planning of the treatment We conclude with an example Karin was to be given special cytostatic treatment The time for this happened to coincide with her sixteenth birthday When told she protested I don t wish to spend my last birthday in hospital The young ward doctor said Be sensible please Karin who was Danish and 6 feet tall got up and looked down on the doctor She said At appellere til fornuften er verdens største slag i luften (Piet Hein) (I e To appeal to the senses is the world s greatest blow in the air)

ACKNOWLEDGEMENT

Translated by Mr W F Salisbury

REFERENCES

1 Bellman M Children and death *Lakartidningen 68* 5555 1971
2 Bennholdt Thomsen C Sterben und Tot des Kindes *Dtsch Med Wschr 84* 1437 1959
3 Burton L *Care of the child facing death* Routledge & Kegan Paul London 1974
4 Easson W M *The dying child The management of the child or adolescent who is dying* C C Thomas Publ Springfield Ill 1970
5 Feigenberg L Death and dying *Lakartidningen 68* 5811 5965 6103 1971
6 Feigenberg L *Terminal care A method for psychological care of dying cancer patients* Thesis Liber laromedel Lund 1977
7 Furman R A Death and the young child *Psychoanal Stud Child 19* 321 1964
8 Johansson B & Larsson G B *Barns tankar om doden* Natur och Kultur Stockholm 1976
9 Koocher G P Talking with children about death *Am J Orthopsychiat 44* 404 1974
10 Morrissey J E A note on interviews with children facing imminent death *Social Casework* 343 1963
11 Nagy M The child s theories concerning death *J Genet Psychol 73* 3 1948
12 Natterson J M Knudson A G Jr Observations concerning fear of death in fatally ill children and their mothers *Psychosom Med 22* 456 1960
13 Roach N *The last day of April* California division —American Cancer Society 1974
14 Solnit A J The dying child *Develop Med Child Neurol 7* 693 1965
15 Spinetta J J Rigler D & Karon M Anxiety in the dying child *Pediatrics 52* 841 1973
16 Timmons A I Leucemia! Is it so awful? *J Pediatr 88* 147 1976

Submitted March 22 1979
Accepted March 22 1979

(I N L) Department of Paediatrics
Malmo Allmanna Sjukhus
S 21401 Malmo
Sweden

KETOTIC HYPOGLYCEMIA OF CHILDHOOD—A CLINICAL TRIAL OF SEVERAL UNIFYING ETIOLOGICAL HYPOTHESES

G DAHLQUIST J GENTZ L HAGENFELDT A LARSSON H LÖW B PERSSON and R ZETTERSTRÖM

From the Department of Paediatrics St Göran s Children s Hospital the Department of Clinical Chemistry and the Department of Endocrinology Karolinska Hospital Stockholm Sweden

ABSTRACT Dahlquist G Gentz J Hagenfeldt L Larsson A Low H Persson B and Zetterstrom R (Department of Paediatrics St Goran s Children s Hospital the Department of Clinical Chemistry and the Department of Endocrinology Karolinska Hospital Stockholm Sweden) Ketotic hypoglycemia of childhood—a clinical trial of several unifying etiological hypotheses Acta Paediatr Scand 68 649 1979 —We have studied 15 children referred to St Goran s Children s Hospital because of suspected ketotic hypoglycemia The patients were investigated according to a program designed to test several hypotheses—old and new—postulated to explain the etiology of ketotic hypoglycemia We have used the classical ketogenic provocation with a low calorie high fat diet and measured the blood levels of several substrates and hormones as well as the urinary excretion of certain metabolites and hormones Out of the 15 children 6 will fill the criteria of ketotic hypoglycemia at the time of study The most remarkable finding in these 6 children in contrast to the other children studied was that they did not decrease their peripheral glucose utilization (measured as Kg) during starvation These 6 children seemed to be more advanced in their adaptation to ketogenic diet in all other parameters studied The children with ketotic hypoglycemia did not differ from the other children in plasma level of cortisol or urinary excretion of nitrogen urea 3-methylhistidine and catecholamines We favour the concept that the children with ketotic hypoglycemia represent the tail of the gaussian curve in the normal age-dependent development of the adaptation to starvation

KEY WORDS Idiopathic hypoglycemia ketotic hypoglycemia childhood hypoglycemia

The most common form of symptomatic hypoglycemia of childhood as pointed out by McQuarrie et al in 1954 (13) is still idiopathic Many of these cases can be classified as the syndrome of ketotic hypoglycemia as defined by Colle & Ulstrom (7) with recurrent episodes of symptomatic hypoglycemia associated with ketonuria occurring after a period of low carbohydrate intake Predominantly children 1 5 to 7 years old are affected and the condition remits spontaneously with growing age The ethiology of this syndrome is still unknown The following hypotheses have been postulated (*a*) a primary defect in the catecholamine response to hypoglycemia (3 18 20) (*b*) a primary defect in the muscle protein catabolism during starvation leading to a defect availability of gluconeogenic substrates mainly in alanine (10 14 18) and (*c*) a primary defect in the cortisol response during hypoglycemia (7)

Which of the ethiological hypotheses indicated above is true remains to be established—but until then the clinical management and diagnostic procedure in suspected cases of ketotic hypoglycemia has to consider several ethiological principles We have therefore studied a consecutive number of such cases admitted to our clinic All parents had given their informed consent for the study The children were investigated before and after provocation with a low calorie–high fat diet mainly with respect to 1) peripheral glucose utilization as measured by intravenous glucose tolerance test 2) muscular protein catabolism as indicated by plasma alanine concentration

Table 1 *Patient data*

		History						Present study			
		Perinatal data									
Case No	Sex	Body weight (g) Length (cm) Gestational age (w)	SGA	Neonatal symptomatic hypoglycemia	Symptoms and signs of ketotic hypoglycemia	Age at onset (y)	Age at last attack (y)	Age (y)	Weight* (SD)	Length* (SD)	Symptomatic response to ketotic diet
Group I											
1	F	2 170 44 37	+	–	Severe vomiting unconscious 3 times	2 7/12	6 4/12	6 5/12	−0 5	−1	+
2	F	1 200 34 37	+	–	Convulsions only one attack	6	6	6 3/12	−1	−0 5	+
3	M	2 960 49 38	–	–	Convulsions only one attack	2 4/12	2 4/12	2 8/12	+1	+1	+
4	M	2 700 50 40	–	+	Unconscious or convulsions	2 11/12	3 3/12	3 6/12	−0 7	+0 2	+
5	M	1 840 44 37	+	+	Unconscious 3 times	2 9/12	3 9/12	3 10/12	−0 7	−0 5	+
6	M	2 000 44 38	+	+	Convulsions 3 times	1 8/12	1 11/12	2 2/12	−1 7	−1 8	+
Group II											
7	M	3 450 50 41	–	–	Recurrent episodes severe vomit tractable with glucose never hypoglyc	3	6 7/1	6 8/2	+1	+0 5	(+)
8	F	3 630 51 41	–	–	Recurrent episodes severe vomit only one low BS value	3	6 3/12	6 7/12	−1 7	−1 8	(+)
9	M	3 430 49 41	+	–	Convulsions only one attack	7d/1	7 4/12	7 9/12	−1 5	−1 2	(+)
Group III											
10	M	3 500 49 43	–	–	No symptoms Dizygotic twin of No 9	–	–	7 /2	+0 8	−0 5	–
11	F	3 500 49 43	–	–	Unconscious twice (pos) ket diettest	1 1/1	6	9/2	−0 7	−2 1	–
12	F	1 760 43 37	+	–	Unconscious twice freq attacks of morn vomit	1	3 2/12	9 3/12	−1 2	−1 0	–
13	F	2 030 44 37	+	–	No symptoms sister of No 12	–	–	12 3/12	−1	+0 1	–

Table 1 *Patient data*

		History						Present study			
		Perinatal data									
Case No	Sex	Body weight (g) Length (cm) Gestational age (w)	SGA	Neonatal symptomatic hypoglycemia	Symptoms and signs of ketotic hypoglycemia	Age at onset (y)	Age at last attack (y)	Age (y)	Weight[a] (SD)	Length[a] (SD)	Symptomatic response to ketonic diet
14	F	3 680 50 41	–	–	Unconscious twice no ver hypoglyc	$3^{2}/_{2}$	$4^{1}/_{12}$	$4\ /_{2}$	+0 2	+0 2	–
15	F	2 850 49 40	–	–	Convulsions only one attack	$/_{2}$	$^{8}/_{12}$	$1\ /_{12}$	−0 1	+0 5	–

Small for gestational age = weight less than −2 SD in relation to gestational age (Sterky G Pediatrics 45 7 1970)
[a] Swedish standards according to Karlberg et al (Acta Paediatr Scand suppl 258 1976)
+ = symptoms (i e vomiting palor) and blood glucose value <2 mmol/l within 24 hours (+) = symptoms but blood glucose >2 mmol/l within 24 hours – = no symptoms blood glucose >2 mmol/l

and urinary excretion of 3 methylhistidine (21 22)) and nitrogen and 3) catecholamine response as measured by urinary catecholamine excretion

MATERIALS AND METHODS

Patients

Fifteen children aged 1 5 to 12 years were studied (Table 1) Eleven of them had a history with one or more episodes of symptomatic hypoglycemia (i e symptoms typical for hypoglycemia associated with a blood glucose value less than 2 mmol/l and symptom relief by glucose) associated with ketonuria occurring after a short period of low energy intake Fasting levels of blood glucose were normal between attacks Glucagon test after 12 hours of fasting was followed by normal blood sugar rise and all children had normal leucine tolerance test Their history and physical examination revealed no known endocrinological or metabolic disease to explain their hypoglycemia Two children (cases 7 and 8) had recurrent episodes of severe vomiting and ketonuria and they responded favourably to intravenous glucose infusion in spite of normal blood glucose concentration The diagnosis ketotic hypoglycemia was therefore suspected in these children Finally two children without symptomatic hypoglycemia were included in the study case 13 i e the healthy sibling of case 1 who had had frequent attacks of hypoglycemia since the neonatal period The mother had severe toxemia during both pregnancies and at birth both girls were small for gestational age Secondly case 10 was the healthy dizygous twin of case 9 who had two episodes of hypoglycemia after 6 years of age They were born at term case 10 with normal birth weight case 9—small for gestational age (Table 1) The two asymptomatic children were included because they might give interesting information about the influence of intrauterine growth retardation on the adaptation to starvation

Procedure Children included in this study had been free of hypoglycemic symptoms and infectious disease for at least 2 weeks before admission to the hospital During the first 3 days after admission the children were fed individually designed diets with the following energy content 50% carbohydrate 20% protein and 30% fat The intake was carefully analysed On the second day after admission an intravenous glucagon test was performed (30 μg of glucagon/kg body weight) and on the third day an intravenous glucose tolerance test (0 5 g/kg body weight) Venous blood samples were drawn at 8 00 a m on the second and third day after admission in the fasting state

On the fourth day the children were given a ketogenic diet as described by Colle & Ulstrom (7) containing 5 040 kJ per 1 73 m² body surface and day 15% carbohydrate 17% protein and 68% fat The diet was provided in 3 meals at 8 00 and 12 00 a m and 5 00 p m

Venous blood samples were collected from an indwelling catheter in the femoral vein starting from 8 00 a m of the foruth day and subsequently every 2 hours until the test was terminated Criteria for termination were either symptomatic hypoglycemia or one blood glucose concentration below 1 5 mmol/l or two repeated values of 2 0 mmol/l or less The test was concluded by another intravenous glucose tolerance test The blood samples collected in heparinized tubes were immediately centrifuged and the plasma was frozen until analysed for glucose β-hydroxybutyrate glycerol alanine insulin and cortisol Urine was collected for 24 hours during the first and the second day after admission and furthermore during the provocation test The urine samples were frozen until analysed for total nitrogen urea nitrogen catecholamine and 3-methylhistidine

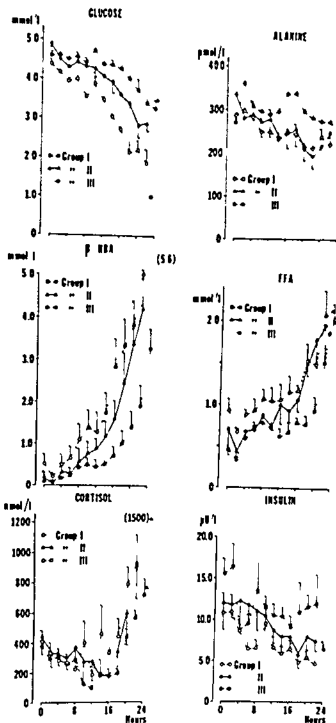


Fig. 1 Plasma concentrations of substrates and hormones during provocation with ketogenic diet in Group I (6 children who developed symptomatic hypoglycemia) Group II (3 children who developed symptoms but remained normoglycemic) and Group III (6 children who remained asymptomatic and normoglycemic) Mean ± S E M is given for each parameter

ANALYTICAL METHODS

Plasma glucose was determined by the glucose oxidase method (GLOX AB* Pharmacia Uppsala Sweden) 3 hydroxybutyrate (15) and glycerol (17) were determined by enzymatic fluorometric techniques Insulin was measured by a standard radioimmunological test system (10) Alanine was estimated by a microfluorometric modification of the method described by Williamson et al (20) Plasma cortisol was measured by radioimmunoassay (11) Urinary catecholamine excretion was determined by a fluorescence method (1) Urea nitrogen and total nitrogen was analysed by standard techniques (Kjeldahl) Urinary 1 methyl histidine and 3 methylhistidine was analysed on the short column of a Beckman* Unichrom amino acid analyser

Statistical methods Wilcoxon s nonparametric tests for both paired and not paired observations were used When p was less than 0 05 differences were considered to be statistically significant

RESULTS

Six children became hypoglycemic (blodo glucose <2 mmol/l) during the provocative test and 5 of them also had symptoms of hypoglycemia (see Table 1) These six children will be referred to as Group I The mean age in this group was 4 /t years

Three children (Group II) developed symptoms in the form of repeated vomiting within 24 hours during the provocative test but they remained normoglycemic and finally 6 children were asymptomatic and normoglycemic and they will be referred to as Group III The mean ages in Group II and III were 7 and 7⁶/₁ years respectively

Group III thus consisted of 3 children who had never had symptomatic hypoglycemia and 3 children with symptomatic hypoglycemia 1–6 years before the present investigation All children in Group I had experienced an episode of symptomatic hypoglycemia within 5 months before admission

Seven of the 11 children with a history of symptomatic hypoglycemia were born small for gestational age (weight less than −2 S D in relation to gestational age according to Swedish standards (19)) Five of the 11 patients with one or more episodes of hypoglycemia had a history of neonatal hypoglycemia (Table 1)

Metabolic response to the ketogenic provocation

Blood glucose levels in Groups I II and III before and during the provocative test are

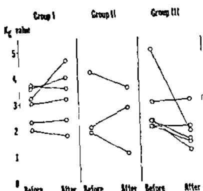


Fig 2 Kg values before and after provocation with ketogenic diet Group I=6 children who developed symptomatic hypoglycemia Group II=3 children who developed symptoms but remained normoglycemic Group III=6 children who remained asymptomatic and normoglycemic

shown in Fig 1 Plasma alanine during the provocation test tended to be higher in Grup II than in Groups I and II (Fig 1) Plasma concentration of FFA glycerol and β hydroxy butyrate tended to be higher in Group I than in Groups II and III (Fig 1) Insulin levels tended to be lower and plasma cortisol higher in Group I compared to the Groups II and III (Fig 1)

Glucose disappearance rate expressed as Kg values decreased significantly during the ketogenic provocation in Group III ($p<0\ 025$) but not in Groups I and II and kg values after ketogenic provocation were significantly lower in Group III than in Group I

Insulin response during the intravenous glucose tolerance test was measured before and immediately after the ketogenic provocation in cases 3 4 5 and 6 of Group I and in cases 7 and 9 in Group II and case 11 in Group III The insulin response was similar in all patients both before and after the ketogenic provocation

The increase in urinary excretion of epinephrine during the provocative test was similar in all groups (Table 2) Urinary norepinephrine excretion did not change significantly during the detogenic provocative test in either group (Table 2) The urinary excretion of Kjeldahl nitrogen urea and 3 methylhistidine decreased significantly during the provocation test in Groups I and III ($p<0\ 03$ and $p<0\ 02$ respectively) The urinary excretion of 3 methylhistidine before the ketogenic provocation was significantly lower in Group III compared to Group I ($p<0\ 025$)

DISCUSSION

In the present study the most striking difference between children of Group I (i e with positive provocation) and Group III (i e with negative provocation) was that in Group I the rate of disappearance of intravenously administered glucose increased or remained unchanged during the ketogenic provocation whereas in Group III it decreased significantly A decrease in glucose disappearance rate has been shown to occur in obese children as well as in obese women during starvation (4 16) Our data on glucose disappearance indicate that children with ketotic hypoglycemia had a limited ability to decrease glucose oxidation during the ketogenic provocation in spite of a normal decrease in circulating insulin Similar results were obtanied by Kerr et al (11) in a study where ^{13}C glucose was used to study glucose turnover in monozygotic twins the smaller of whom fulfilled the diagnostic criteria for ketotic hypoglycemia Failure to decrease glucose utilization in response to fasting or a ketogenic diet thus seems to be the main cause of hypoglycemia also in these children In this context it is interesting to note that during starvation 20 days old intrauterine growth retarded rats were shown to be more dependent on glucose as a substrate for the brain as they had a significantly lower cerebral utilization of ketone bodies compared to normal litter mates (8)

The increase in urinary epinephrine excretion during the provocative test was similar in Groups I and II There was thus no indication that a diminished catecholamine response contributed to the *development* of hypoglycemia On the other hand the epinephrine

ACKNOWLEDGEMENTS

This study was supported by grants from Semper Foundation for Nutritional Research Stiftelsen Samariten and Thielman s Foundation

REFERENCES

1 Andersson B Hovmuller S Karlsson C G & Svensson S Analysis of urinary catecholamines by an improved auto-analyser fluorescence method *Reports from the Laboratory of Clinical Stress Research* Departments of Medicine and Psychiatry Karolinska Sjukhuset 32 1973

2 Bier D M Leake R D Haymond M W Arnold K J Gruenke L D Sperling M A & Kipnis D M Measurement of true glucose production rates of infancy and childhood with 6 6-dideuteroglucose *Diabetes 26* 1016 1977

3 Broberger O & Zetterstrom R Hypoglycemia with an inability to increase the epinephrine secretion in insulin induced hypoglycemia *J Pediatr 56* 215 1961

4 Cahill G Herrera M G Morgan A P Soeldner J S Steinke J Levy P L Reichard G A & Kipnis D M Hormone fuel interrelationship during fasting *J Clin Invest 45* 1751 1966

5 Chaussin J L Georges P Calzada L & Job J C Glycemic response to 24 hour fast in normal children III Influence of age *J Pediatr 95* 711 1977

6 Christensen N J Hypoadrenalinemia during insulin hypoglycemia in children with ketotic hypoglycemia *J Clin Endocrinol Metab 38* 107 1974

7 Colle E & Ulstrom R A Ketotoc hypoglycemia *J Pediatr 64* 632 1964

8 Dahlquist G Cerebral utilization of glucose ketone bodies and oxygen in starving infant rats and the effect of intrauterine growth retardation *Acta Physiol Scand 98* 237 1976

9 Habbrick B F McNeish A S & Stephenson J B P Diagnosis of ketotic hypoglycemia of childhood *Arch Dis Child 46* 295 1971

10 Haymond M W Karl J E & Pagliara A S Ketotic hypoglycemia an aminoacid substrate limited disorder *J Clin Endocrinol Metab 38* 521 1974

11 Kerr D S Stevens M C G Robinson H M & Picon D J M Hypoglycemia and the regulation of the fasting glucose metabolism in malnutrition *In* L J Gardner & P Amacher (eds) *Endocrine aspects of malnutrition* The Kroc Foundation Santa Yuez 1973

12 Laurell S & Tibbling G An enzymatic fluorometric micromethod for the determination of glycerol *Clin Chim Acta 13* 317 1966

13 McQuarrie I Ulstrom R A & Ziegler M R Random notes concerning the ethiological mechanisms and treatment of spontaneous hypoglycemia *Acta Paediatr Scand 43* Suppl 100 1954

14 Pagliara A S Karl J E De Vivo D C Feigin R D & Kipnis D M Hypoalaninemia a concomitant of ketotic hypoglycemia *J Clin Invest 51* 1440 1972

15 Persson B Determination of plasma acetoacetate and D β hydroxy butyrate in newborn infants by an enzymatic fluorometric micromethod *Scand J Clin Lab Invest 29* 9 1970

16 Persson B Sterky G & Thorell J Effect of low carbohydrate diet on plasma glucose free fatty acid glycerol ketones and insulin during glucose tolerance tests in adolescent boys *Metabolism 16* 14 1967

17 Ruder H J Guy R L & Lipsett M B A radioimmunoassay for cortisol in plasma and urine *J Clin Endocrinol Metab 35* 219 1972

18 Sizonenko P L Paunier L Vallotton M B Cuendet G Z & Marliss E B Response to deoxy D-glucose and to glucogen in ketotic hypoglycemia of childhood evidence for epinephrine deficiency and altered alanine availability *Pediatr Res 7* 983 1973

19 Sterky G Swedish standard curves for intrauterine growth *Pediatrics 45* 7 1970

20 Williamson P H Lopez Vieira D & Walker B Concentrations of free gluconeogenic amino acids in livers of rats subjected to various metabolic stresses *Biochem J 104* 497 1967

21 Young V R Alexis S D Baliga B S & Munro H N Metabolism of administered 3 methylhistidine *J Biol Chem 247* 3592 1971

22 Young V R Haverberg L N Bilmazes C & Munro H N Potential use of 3 methylhistidine excretion as an index of progressive reduction in muscle protein catabolism during starvation *Metabolism 22* 1429 1973

Submitted Dec 12 1978
Accepted Febr 27 1979

(G D) Department of Paediatrics
St Goran s Children s Hospital
Box 12500
S 112 81 Stockholm
Sweden

DO PRE AND POSTCHALLENGE SMALL INTESTINAL BIOPSIES HELP TO DIAGNOSE COW S MILK PROTEIN INTOLERANCE?

N O BERG I JAKOBSSON and T LINDBERG

From the Department of Paediatric Malmo General Hospital Malmo and the Department of Pathology University of Lund Sweden

ABSTRACT Berg N O Jakobsson I and Lindberg T (Department of Paediatrics Malmo General Hospital and Department of Pathology University of Lund Sweden) Do pre and postchallenge small intestinal biopsies help to diagnose cow s milk protein intolerance? Acta Paediatr Scand 68 657 1979.—Twelve infants suspected of cow s milk protein intolerance were challenged with cow s milk after at least one month of cow s milk free diet The challenge was clinically positive in seven Small intestinal biopsies were taken with a multipurpose capsule both pre and postchallenge (at 24 h) in eight prechallenge in three and postchallenge in one Two or three biopsy specimens were taken at the same time in 15 of the 20 biopsy occasions The morphology of the mucosa could vary from normal to slight damage at the same biopsy occasion No difference was found in morphology judged by light microscopy between pre and postchallenged biopsies Light microscopy of small intestinal biopsies taken before and at 24 h after milk challenge seems of doubtful value as a routine diagnostic means in cow s milk protein intolerance

KEY WORDS Cow s milk protein intolerance small intestine small intestinal biopsy

The study of the clinical response to elimination and reintroduction of cow s milk has long been the only way to diagnose cow s milk protein intolerance (5) The small intestinal mucosa is damaged in variable degree in this disease and shows no characteristic changes (4 10 12 17 19 21) Histological ultrastructural and immunological changes of small intestinal mucosa after challenge with cow s milk have been reported (6 7 9 11 12 14 15 17 18 20 21) The value of routine light microscopy of pre and postchallenge (at 24 h) small intestinal biopsies for the diagnosis of cow s milk protein intolerance has been discussed (3 6 15 16 17 18 20) The patchiness of the mucosal lesions (17 20 21) perhaps limits the value of this method

To evaluate this procedure as a routine diagnostic means we studied the morphology in multiple biopsies from twelve infants with suspected cow s milk protein intolerance Eight were biopsied both before and after challenge with cow s milk three before and one after The results are reported here

PATIENTS AND METHODS

Twelve infants were studied seven had an heredity to allergic diseases Symptoms (*diarrhoea vomiting* infantile colic and/or failure to thrive) began at age 2 weeks to 6 months In eight the symptoms appeared within one month on a cow s milk containing formula Three developed symptoms on breast *milk and* were free of symptoms with the mothers on a cow s milk *free diet* (8) Lactose intolerance was excluded

All became symptomfree and gained weight when their *diet* was changed to a strict cow s milk free diet (soy formula Nutramigen® (Mead Johnson) or cow s milk free breast milk) After at least 4 weeks *on this diet* they were challenged with cow s milk After premedication with chloralhydrate a prechallenge biopsy was taken with a hydraulic capsule (13) at the duodeno jejunal *flexure* under fluoroscopic control

The infants were then given increasing amounts of cow s milk *(1 ml to 50 ml)* every third hour and two 50 ml doses of cow s milk 12 *hours and* 2 hours before biopsy A symptomfree infant had a total of 1 *6 ml of* cow s milk When clinical signs appeared the symptom giving *dose* was *repeated* once more The postchallenge biopsy was taken from the same *region as the* first one 24 hours after the challenge began *Two or three* specimens were usually taken a few centimeters apart

The biopsy specimens were oriented on a millipore filter and fixed in 4% *formol solution* with short after fixation in Bouin s solution They *were then* examined and photographed in a dissecting microscope *After* embedding in paraffin or plastic the biopsy was serially cut into 5–6 μm

ACKNOWLEDGEMENTS

This study was supported by grants from Semper Foundation for Nutritional Research Stiftelsen Samariten and Thielmans Foundation

REFERENCES

1 Andersson B Hovmuller S Karlsson C G & Svensson S Analysis of urinary catecholamines by an improved auto-analyser fluorescence method *Reports from the Laboratory of Clinical Stress Research* Departments of Medicine and Psychiatry Karolinska Sjukhuset 32 1973
2 Bier D M Leake R D Haymond M W Arnold K J Gruenke L D Sperling M A & Kipnis D M Measurement of true glucose production rates of infancy and childhood with 6 6-dideuteroglucose *Diabetes 26* 1016 1977
3 Broberger O & Zetterström R Hypoglycemia with an inability to increase the epinephrine secretion in insulin induced hypoglycemia *J Pediatr 56* 215 1961
4 Cahill G Herrera M G Morgan A P Soeldner J S Steinke J Levy P I Reichard G A & Kipnis D M Hormone fuel interrelationship during fasting *J Clin Invest 45* 1751 1966
5 Chaussin J L Georges P Calzada L & Job J C Glycemic response to 24 hour fast in normal children III Influence of age *J Pediatr 95* 711 1977
6 Christensen N J Hypoadrenalinemia during insulin hypoglycemia in children with ketotic hypoglycemia *J Clin Endocrinol Metab 38* 107 1974
7 Colle E & Ulstrom R A Ketotoc hypoglycemia *J Pediatr 64* 632 1964
8 Dahlquist G Cerebral utilization of glucose ketone bodies and oxygen in starving infant rats and the effect of intrauterine growth retardation *Acta Physiol Scand 98* 237 1976
9 Habbrick B F McNeish A S & Stephenson J B P Diagnosis of ketotic hypoglycemia of childhood *Arch Dis Child 46* 295 1971
10 Haymond M W Karl J E & Pagliara A S Ketotic hypoglycemia an aminoacid substrate limited disorder *J Clin Endocrinol Metab 38* 521 1974
11 Kerr D S Stevens M C G Robinson H M & Picon D J M Hypoglycemia and the regulation of the fasting glucose metabolism in malnutrition *In* L J Gardner & P Amacher (eds) *Endocrine aspects of malnutrition* The Kroc Foundation Santa Yuez 1973
12 Laurell S & Tibbling G An enzymatic fluorometric micromethod for the determination of glycerol *Clin Chim Acta 13* 317 1966
13 McQuarrie I Ulstrom R A & Ziegler M R Random notes concerning the ethiological mechanisms and treatment of spontaneous hypoglycemia *Acta Paediatr Scand 43* Suppl 100 1954
14 Pagliara A S Karl J F De Vivo D C Feiger R D & Kipnis D M Hypoalaninemia a concomitant of ketotic hypoglycemia *J Clin Invest 51* 1440 1972
15 Persson B Determination of plasma acetoacetate and D β hydroxy butyrate in newborn infants by an enzymatic fluorometric micromethod *Scand J Clin Lab Invest 29* 9 1970
16 Persson B Sterky G & Thorell J Effect of low carbohydrate diet on plasma glucose free fatty acids glycerol ketones and insulin during glucose tolerance tests in adolescent boys *Metabolism 16* 14 1967
17 Ruder H J Guy R L & Lipsett M B A radioimmunoassay for cortisol in plasma and urine *J Clin Endocrinol Metab 35* 219 1972
18 Sizonenko P L Paunier L Vallotton M B Cuendet G Z & Marliss E B Response to 2 deoxy D-glucose and to glucogen in ketotic hypoglycemia of childhood evidence for epinephrine deficiency and altered alanine availability *Pediatr Res 7* 983 1973
19 Sterky G Swedish standard curves for intrauterine growth *Pediatrics 45* 7 1970
20 Williamson P H Lopez Vieira D & Walker B Concentrations of free gluconeogenic amino acids in livers of rats subjected to various metabolic stresses *Biochem J 104* 497 1967
21 Young V R Alexis S D Baliga B S & Munro H N Metabolism of administered 3 methylhistidine *J Biol Chem 247* 3592 1971
22 Young V R Haverberg L N Bilmazes C & Munro H N Potential use of 3 methylhistidine excretion as an index of progressive reduction in muscle protein catabolism during starvation *Metabolism 22* 1429 1973

Submitted Dec 12 1978
Accepted Febr 27 1979

(G D) Department of Paediatrics
St Goran s Children s Hospital
Box 12500
S 112 81 Stockholm
Sweden

Acta Pædiatr Scand 68 657–661 1979

DO PRE AND POSTCHALLENGE SMALL INTESTINAL BIOPSIES HELP TO DIAGNOSE COW S MILK PROTEIN INTOLERANCE?

N O BERG I JAKOBSSON and T LINDBERG

From the Department of Paediatric Malmo General Hospital Malmo and the Department of Pathology University of Lund Sweden

ABSTRACT Berg N O Jakobsson I and Lindberg T (Department of Paediatrics Malmo General Hospital and Department of Pathology University of Lund Sweden) Do pre and postchallenge small intestinal biopsies help to diagnose cow s milk protein intolerance Acta Paediatr Scand 68 657 1979 —Twelve infants suspected of cow s milk protein intolerance were challenged with cow s milk after at least one month of cow s milk free diet The challenge was clinically positive in seven Small intestinal biopsies were taken with a multipurpose capsule both pre and postchallenge (at 24 h) in eight prechallenge in three and postchallenge in one Two or three biopsy specimens were taken at the same time in 15 of the 20 biopsy occasions The morphology of the mucosa could vary from normal to slight damage at the same biopsy occasion No difference was found in morphology judged by light microscopy between pre and postchallenged biopsies Light microscopy of small intestinal biopsies taken before and at 24 h after milk challenge seems of doubtful value as a routine diagnostic means in cow s milk protein intolerance

KEY WORDS Cow s milk protein intolerance small intestine small intestinal biopsy

The study of the clinical response to elimination and reintroduction of cow s milk has long been the only way to diagnose cow s milk protein intolerance (5) The small intestinal mucosa is damaged in variable degree in this disease and shows no characteristic changes (4 10 12 17 19 21) Histological ultrastructural and immunological changes of small intestinal mucosa after challenge with cow s milk have been reported (6 7 9 11 12 14 15 17 18 20 21) The value of routine light microscopy of pre and postchallenge (at 24 h) small intestinal biopsies for the diagnosis of cow s milk protein intolerance has been discussed (3 6 15 16 17 18 20) The patchiness of the mucosal lesions (17 20 21) perhaps limits the value of this method

To evaluate this procedure as a routine diagnostic means we studied the morphology in multiple biopsies from twelve infants with suspected cow s milk protein intolerance Eight were biopsied both before and after challenge with cow s milk three before and one after The results are reported here

PATIENTS AND METHODS

Twelve infants were studied seven had an heredity to allergic diseases Symptoms (diarrhoea vomiting infantile colic and/or failure to thrive) began at age 2 weeks to 6 months In eight the symptoms appeared within one month on a cow s milk containing formula Three developed symptoms on breast milk and were free of symptoms with the mothers on a cow s milk free diet (8) Lactose intolerance was excluded

All became symptomfree and gained weight when their diet was changed to a strict cow s milk free diet (soy formula Nutramigen® (Mead Johnson) or cow s milk free breast milk) After at least 4 weeks on this diet they were challenged with cow s milk After premedication with chloralhydrate a prechallenge biopsy was taken with a hydraulic capsule (13) at the duodeno jejunal flexure under fluoroscopic control

The infants were then given increasing amounts of cow s milk (1 ml to 50 ml) every third hour and two 50 ml doses of cow s milk 12 hours and 2 hours before biopsy A symptomfree infant had a total of 126 ml of cow s milk When clinical signs appeared the symptom giving dose was repeated once more The postchallenge biopsy was taken from the same region as the first one 24 hours after the challenge began Two or three specimens were usually taken a few centimeters apart

The biopsy specimens were oriented on a millipore filter and fixed in 4% formol solution with short after fixation in Bouin s solution They were then examined and photographed in a dissecting microscope After embedding in paraffin or plastic the biopsy was serially cut into 5–6 μm

Table 1 *Clinical and morphological results of milk challenge in cow s milk protein intolerance*

Patient	Cow s milk introduced age (d)	Onset of symptoms age (d)	Cow s milk free diet age (d)	Milk challenge age (d)	Total amounts of milk (ml)	Clinical results	Morphological results: Prechallenge Villi	Prechallenge Histology	Postchallenge Villi	Postchallenge Histology
P O ♀	7	24	75	150	126	Positive[a] diarrhoea	(a) Leaves+ridges (b) Leaves	SL SL	(a) Leaves+ridges (b) Leaves+ridges	SL N?
T J ♀	30	30	45	120	11	Positive vomiting diarrhoea	(a) Leaves+ridges (b) Ridges	SL SL	(a) Leaves+ridges (b) Leaves+ridges	SL SL
L Å ♀	30	60	120	150	126	Positive[a] vomiting diarrhoea	(a) Leaves (b) Leaves	SL SL	Leaves+ridges	SL
F A ♂	180	180	210	240	126	Positive diarrhoea	(a) Leaves+ridges (b) Leaves	SL SL	(a) Ridges (b) Ridges (c) Leaves+ridges	N? SL SL
A J ♀	2	60	120	180	126	Positive colic diarrhoea	(a) Ridges (b) Ridges (c) Ridges	N? N SL	– –	– –
C A ♀	115	115	150	235	11	Positive vomiting	(a) Ridges (b) Not evaluable	SL SL	– –	– –
O T ♂	–	30	30	90	11	Positive colic diarrhoea	–	–	(a) Finger+Leaves (b) Leaves	N? N?
M G[c] ♂	–	14	45	90	126	Negative	Ridges	SL	Leaves+ridges	SL
A K[c] ♀	–	45	45	180	126	Negative	Finger+leaves	N?	(a) Leaves (b) Leaves	SL SL
F F ♂	30	60	75	150	126	Negative	(a) Not evaluable (b) Ridges	SL SL	(a) Ridges (b) Ridges	SL SL
J P ♂	45	60	75	180	126	Negative	(a) Leaves (b) Leaves+ridges	N? SL	Ridges	SL
I F ♂	120	130	150	225	126	Negative	(a) Leaves (b) Leaves+ridges	N? SL	–	–

N=normal N?=uncertain slight cell infiltration otherwise normal epithelial cells normal SL=slight damage in epithelial lymphocytes distinct foci of plasma cells and/or leucocytes (mostly eosinophils) slight elongation of the crypt
[a] 2–4 days after challenge
[c] Breastfed infants with severe colic disappearing with the mother on a cow s milk free diet

sections and 1–2 μm sections respectively The sections were stained with Htxl Eosin PAS according to Mc Manus and Giemsa stain for mast cells The best oriented central cores of the specimens were used for assessment The specimens were examined without knowledge of clinical findings

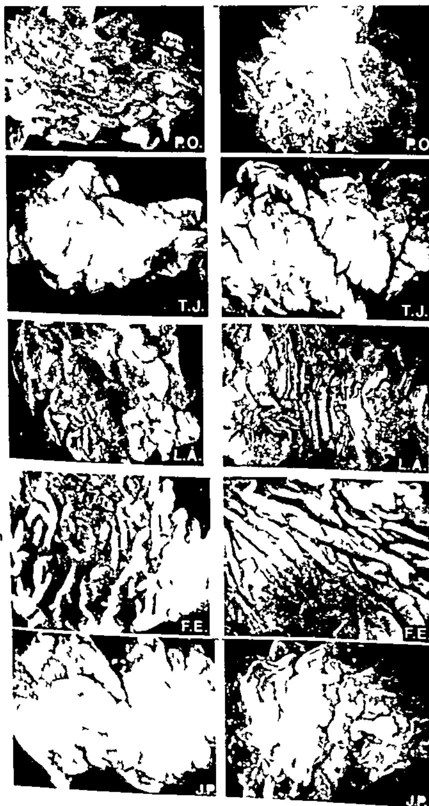
Infants with symptoms on challenge had a cow s milk free diet Symptomfree infants continued to have a cow s milk containing formula at home and were controlled regularly If no symptoms reappeared within twelve months the challenge was judged negative All the infants were followed for at least one year after the challenge

RESULTS

Table 1 gives some clinical data and the clinical and morphological results of the challenge

The challenge was considered clinically positive in seven infants two of whom had no symptoms until 2–4 days after the challenge A cow s milk free formula was successfully reintroduced in all seven They were later rechallenged with positive results but at one year of age all except two tolerated a normal

Fig 1 Appearance of intestinal mucosa in the dissecting microscope before (to the left) and after (to the right) milk challenge in five children For P O T J and L Å the challenge was clinically positive but for F E and J P the challenge was negative Slender or broader leaves and slightly increased ridging is seen for P O


P.O.
P.O
T.J.
T.J.
L.A.
L.A.
F.E.
F.E.
J.P.
J.P.

diet These two are still at two years of age on a cow s milk free diet because of eczema The other five with no clinical signs on challenge continued on normal cow s milk containing diet They had no symptoms and had *gained weight normally one two and twelve* months after the challenge

Eight infants were biopsied both before and after the challenge three before and one after Two or three specimens were taken at the same time at 15 out of the 20 biopsy occasions When comparing these double specimens ten showed the same histological picture and five showed slight variations between the specimens (Table 1) There was no difference in mucosal morphology between infants with a positive challenge and those with a negative one (Fig 1 and Table 1) We could not observe any deterioration of the intestinal mucosa after the milk challenge compared with the prechallenged biopsies In particular there was no significant change in the epithelial cells no increase in infiltrating inflammatory cells no evident decrease in number of mast cells and no change in villous/crypt ratio

DISCUSSION

There is a need for an objective and rapid method for diagnosing cow s milk protein intolerance Therefore the reports (6 7 15 17) on even major (6 7 17) changes of morphology in intestinal biopsies 24 h after challenge with cow s milk were promising

The present study found only slight morphological abnormalities of the intestinal mucosa Thus in no case were distinct convolutions seen in the dissecting microscope and in well orientated sections the villous/crypt height ratio was always more than 1 1

The study also revealed the patchy nature of the mucosal lesions (17 20 21) The specimens in 5 of 15 biopsy occasions showed a histological variation from normal to slight damage irrespective of whether the biopsy was taken pre or postchallenge and whether the challenge was clinically positive or negative

We saw no definite changes in dissecting and light microscopy of the small intestinal mucosa at 24 hours after the challenge began compared with the prechallenge morphology irrespective of whether the challenge was clinically positive or negative From the infants with a positive clinical response 13 mucosal specimens were taken on 6 pre and 11 at 5 postchallenge biopsy occasions On the whole there were no more abnormalities in the postchallenge mucosal specimens These findings are in contrast to those of Shiner et al (15) Sumithran & Iyngkaran (17) and Iyngkaran et al (6, 7) The reason for this is perhaps that these authors gave a larger challenge dose of milk and that the infants had more severe symptoms initially In cow s milk protein intolerance there is a varying degree in the tolerance of the mucosa to the cow s milk proteins (12) and the milk dose might be critical for the appearance of mucosal abnormalities It is well known that the reactions can be severe after giving cow s milk to these infants (e g severe diarrhoea requiring intravenous fluids because of dehydration (21)) and we considered that a further increase in the amount of milk was unjustified In our opinion the symptoms and signs presented by the infants in this report are representative for infants with cow s milk protein intolerance in this part of the world The infants were not treated earlier and for a longer time before challenge than in the previous studies (6 7 15 17)

Finally it is debatable whether the infants with a clinically positive challenge really had cow s milk protein intolerance and whether instead the reactions were due to some intercurrent illness However all reacted with similar symptoms at at least two more cow s milk challenges Therefore we are convinced that the infants had cow s milk protein intolerance

From this study it can be concluded that a postchallenge biopsy at 24 h without any histological deterioration does not exclude the possibility of cow s milk protein intolerance and that the clinical result of milk challenge

does not necessarily run parallel with the morphological results judged by routine methods This design of diagnostic procedure seems of doubtful value

Analyses of intestinal disaccharides or dipeptidases may be a more sensitive test of mucosal damage Walker Smith et al (21) found that analyses of intestinal disaccharidases in pre and postchallenge biopsies were a helpful guide to mucosal relapse However these authors did the postchallenge biopsy after more than 48 h on cow s milk and the biopsies showed a significant morphological deterioration It is known that the intestinal disaccharidase and dipeptidase activities are significantly correlated to morphological findings (2) In addition the results of enzyme analyses can be difficult to estimate as the mucosal lesion both pre and postchallenge can be patchy in this disorder

We emphasize that in cow s milk protein intolerance as in other malabsorption syndromes a small intestinal biopsy is the only way to determine the existence of a mucosal lesion (1) Moreover in doubtful cases it is an invaluable means for following the effect of elimination and reintroduction of cow s milk over a longer period (11 21)

ACKNOWLEDGEMENTS

Grants from the Swedish Medical Research Council (project no 5143 and 5364) Albert Påhlsson Foundation Alfred Österlund Foundation The Medical Research Council of the Swedish Life Insurance Companies The Swedish Baby Food Industry Fund for Nutritional Research and Swedish Nutrition Foundation supported this work

REFERENCES

1 Berg N O Borulf S Jakobsson I & Lindberg T How to approach the child suspected of malabsorption *A ta Paediatr Scand* 67 403 1978

Berg N O Dahlqvist A Lindberg T & Norden Å Correlation between morphological alterations and enzyme activities in the mucosa of the small intest ne *Scand J Gastroente ol 8* 703 1973

3 Editorial Cow s milk protein and the small intestine *Lancet I* 70 1978

4 Fontaine J L & Navarro J Small intestinal biopsy in cow s m lk protein allergy in infancy *Arch D s Child* 50 357 1975

5 Goldman A S Andersson D W Jr Sellers W A Saperstein S Kniker W T & Halpern S R Milk allergy I Oral challenge with milk and isolated milk proteins in allergic children *Pediatrics 32* 425 1963

6 Iyngkaran N Robinson M J Prathap K Sumithran E & Yatav M Cow s milk protein sensitive enteropathy *Arch Dis Child 53* 70 1978

7 Iyngkaran N Robinson M J Sumithran E Lam S K Putchucheary S D & Yadav M Cow s milk protein sensitive enteropathy An important factor in prolonging diarrhoea of acute infective enteritis in early infancy *Arch Dis Child 53* 150 1978

8 Jakobsson I & Lindberg T Cow s milk as a cause of infantile colic in breastfed infants *Lancet II* 437 1978

9 Kilby A Walker Smith J A & Wood C B S Small intestinal mucosa in cow s milk allergy *Lancet I* 531 1975

10 Kuitunen P Visakorpi J K & Hallman N Histopathology of duodenal mucosa in malabsorption syndrome induced by cow s milk *Ann Paediat 205* 54 1965

11 Kuitunen P Rapola J Savilahti E & Visakorpi J P Response of the jejunal mucosa to cow s milk intolerance A light and electron microscopic study *Acta Paediatr Scand* 6? 585 1973

12 Kuitunen P Visakorpi J K Savilahti E & Pelkonen P Malabsorption syndrome with cow s milk intolerance Clinical findings and course in 54 cases *Arch Dis Ch ld 50* 351 1975

13 Meuwisse G Lindberg T & Blomberg M A hydraulic multiple intestinal biopsy apparatus for infants *Acta Paediatr Scand 63* 839 1974

14 Shiner M Ballard J & Smith M The small intestinal mucosa in cow s milk allergy *Lancet I* 136 1975

15 Shiner M Ballard J Brook C G D & Herman S Intestinal biopsy in the diagnosis of cow s milk protein intolerance without acute symptoms *Lancet II* 1060 1975

16 Shmerling D H & Rein D Jejunal biopsy in cow s milk protein intolerance *Lancet I* .7? 1978

17 Sumithran E & Iyngkaran N Is jejunal biopsy really necessary in cow s milk protein intolerance? *Lancet II* 11?? 1977

18 Vitoria J C Aranjuelo M E & Rodriguez Soriano J Jejunal biopsy in cow s milk protein intolerance *Lancet I* 7?? 1978

19 Walker Smith J Cow s milk protein intolerance Transient food intolerance of infancy *Arch Dis Child 50* 347 1975

0 Walker Smith J A Jejunal biopsy in cow s milk protein intolerance *Lancet II* 1237 1977

?1 Walker Smith J A Harrison M Kilby B Philips A & France N Cow s milk sensitive enteropathy *Arch Dis Child 53* 375 1978

Submitted Nov ?7 1978
Accepted Febr ?6 1979

(I J) Department of Paediatrics
Malmo General Hospital
S-?1401 Malmö Sweden

diet These two are still at two years of age on a cow s milk free diet because of eczema The other five with no clinical signs on challenge continued on normal cow s milk containing diet They had no symptoms and had gained weight normally one two and twelve months after the challenge

Eight infants were biopsied both before and after the challenge three before and one after Two or three specimens were taken at the same time at 15 out of the 20 biopsy occasions When comparing these double specimens ten showed the same histological picture and five showed slight variations between the specimens (Table 1) There was no difference in mucosal morphology between infants with a positive challenge and those with a negative one (Fig 1 and Table 1) We could not observe any deterioration of the intestinal mucosa after the milk challenge compared with the prechallenged biopsies In particular there was no significant change in the epithelial cells no increase in infiltrating inflammatory cells no evident decrease in number of mast cells and no change in villous/crypt ratio

DISCUSSION

There is a need for an objective and rapid method for diagnosing cow s milk protein intolerance Therefore the reports (6 7 15 17) on even major (6 7 17) changes of morphology in intestinal biopsies 24 h after challenge with cow s milk were promising

The present study found only slight morphological abnormalities of the intestinal mucosa Thus in no case were distinct convolutions seen in the dissecting microscope and in well orientated sections the villous/crypt height ratio was always more than 1 1

The study also revealed the patchy nature of the mucosal lesions (17 20 21) The specimens in 5 of 15 biopsy occasions showed a histological variation from normal to slight damage irrespective of whether the biopsy was taken pre or postchallenge and whether the challenge was clinically positive or negative

We saw no definite changes in dissecting and light microscopy of the small intestinal mucosa at 24 hours after the challenge began compared with the prechallenge morphology irrespective of whether the challenge was clinically positive or negative From the infants with a positive clinical response 13 mucosal specimens were taken on 6 pre and 11 at 5 postchallenge biopsy occasions On the whole there were no more abnormalities in the postchallenge mucosal specimens These findings are in contrast to those of Shiner et al (15) Sumithran & Iyngkaran (17) and Iyngkaran et al (6 7) The reason for this is perhaps that these authors gave a larger challenge dose of milk and that the infants had more severe symptoms initially In cow s milk protein intolerance there is a varying degree in the tolerance of the mucosa to the cow milk proteins (12) and the milk dose might be critical for the appearance of mucosal abnormalities It is well known that the reaction can be severe after giving cow s milk to these infants (e g severe diarrhoea requiring intravenous fluids because of dehydration (21) and we considered that a further increase in the amount of milk was unjustified In our opinion the symptoms and signs presented by the infants in this report are representative for infants with cow s milk protein intolerance in this part of the world The infants were not treated earlier and for a longer time before challenge than in the previous studies (6 7 15 17)

Finally it is debatable whether the infants with a clinically positive challenge really had cow s milk protein intolerance and whether instead the reactions were due to some intercurrent illness However all reacted with similar symptoms at at least two more cow s milk challenges Therefore we are convinced that the infants had cow s milk protein intolerance

From this study it can be concluded that a postchallenge biopsy at 24 h without any histological deterioration does not exclude the possibility of cow s milk protein intolerance and that the clinical result of milk challenge

loes not necessarily run parallel with the mor phological results judged by routine methods This design of diagnostic procedure seems of doubtful value

Analyses of intestinal disaccharides or di peptidases may be a more sensitive test of mucosal damage Walker Smith et al (21) found that analyses of intestinal disacchanid ases in pre and postchallenge biopsies were a helpful guide to mucosal relapse However these authors did the postchallenge biopsy after more than 48 h on cow s milk and the biopsies showed a significant morphological deterioration It is known that the intestinal disaccharidase and dipeptidase activities are significantly correlated to morphological find ings (?) In addition the results of enzyme analyses can be difficult to estimate as the mucosal lesion both pre and postchallenge can be patchy in this disorder

We emphasize that in cow s milk protein intolerance as in other malabsorption syn dromes a small intestinal biopsy is the only way to determine the existence of a mucosal lesion (1) Moreover in doubtful cases it is an invaluable means for following the effect of elimination and reintroduction of cow s milk over a longer period (11 21)

ACKNOWLEDGEMENTS

Grants from the Swedish Medical Research Council (pro ject no 5143 and 5364) Albert Påhlsson Foundation Alfred Österlund Foundation The Medical Research Council of the Swedish Life Insurance Companies The Swedish Baby Food Industry Fund for Nutritional Re search and Swedish Nutrition Foundation supported this work

REFERENCES

1 Berg N O Borulf S Jakobsson I & Lindberg T How to approach the child suspected of malab- sorpt on *Acta Paed atr Scand* 67 403 1978
2 Berg N O Dahlqvist A Lindberg T & Norden A Correlation between morphological alterations and enzyme activities in the mucosa of the small in testine *S a d J Gast oente ol* 8 703 1973
3 Ed torial Cow s milk protein and the small intestine *Lancet I* 70? 1978
4 Fonta ne J L & Navarro J Small intestinal bi opsy in cow s m lk protein allergy in infancy *Arch D s Child* 50 357 1975
5 Goldman A S Anderson D W Jr Sellers W
A Saperstein S Kniker W T & Halpern S R Milk allergy I Oral challenge with milk and isolated milk proteins in allergic children *Pediatrics* 3? 4?5 1963
6 Iyngkaran N Robinson M J Prathap K Su mithran E & Yatav M Cow s milk protein sensi tive enteropathy *Arch Dis Child* 53 20 1978
7 Iyngkaran N Robinson M J Sumithran E Lam S K Puthucheary S D & Yadav M Cow s milk protein sensitive enteropathy An important factor in prolonging diarrhoea of acute infective enteritis in early infancy *Arch Dis Child* 53 150 1978
8 Jakobsson I & Lindberg T Cow s milk as a cause of infantile colic in breastfed infants *Lancet II* 437 1978
9 Kilby A Walker Smith J A & Wood C B S Small intestinal mucosa in cow s milk allergy *Lancet I* 531 1975
10 Kuitunen P Visakorpi J K & Hallman N Histo pathology of duodenal mucosa in malabsorption syn drome induced by cow s milk *Ann Paediat 205* 54 1965
11 Kuitunen P Rapola J Savilathi E & Visakorpi J P Response of the jejunal mucosa to cow s milk intolerance A light and electron microscopic study *Acta Paediatr Scand* 6? 585 1973
12 Kuitunen P Visakorpi J K Savilathi E & Pel konen P Malabsorption syndrome with cow s milk intolerance Clinical findings and course in 54 cases *Arch Dis Child 50* 351 1975
13 Meuwisse G Lindberg T & Blomberg M A hydraulic multiple intestinal biopsy apparatus for in fants *Acta Paediatr Scand 63* 839 1974
14 Shiner M Ballard J & Smith M The small intes tinal mucosa in cow s milk allergy *Lancet I* 136 1975
15 Shiner M Ballard J Brook C G D & Herman S Intestinal biopsy in the diagnosis of cow s milk protein intolerance without acute symptoms *Lancet II* 1060 1975
16 Shmerling D H & Rein D Jejunal biopsy in cow s milk protein intolerance *Lancet I* 272 1978
17 Sumithran E & Iyngkaran N Is jejunal biopsy really necessary in cow s milk protein intolerance? *Lancet II* 112? 1977
18 Vitoria J C Aranjuelo M E & Rodriguez Soriano J Jejunal biopsy in cow s milk protein intolerance *Lancet I* 72? 1978
19 Walker Smith J Cow s milk protein intolerance Transient food intolerance of infancy *Arch Dis Child 50* 347 1975
20 Walker Smith J A Jejunal biopsy in cow s milk protein intolerance *Lancet II* 1?37 1977
21 Walker Smith J A Harrison M Kilby B Philips A & France N Cow s milk sensitive enteropathy *Arch Dis Child* 53 375 1978

Submitted Nov 27 1978
Accepted Febr ?6 1979

(I J) Department of Paediatrics
Malmö General Hospital
S 21401 Malmo Sweden

Acta Pædiatr Scand 68 663–667 1979

A PROSPECTIVE STUDY OF URINARY PROTEINS IN EARLY INFANCY

F ANDERS KARLSSON LARS IVAR HARDELL
and KRISTOFFER HELLSING

From the Departments of Clinical Research Internal Medicine Paediatrics and Clinical Chemistry University Hospital University of Uppsala Uppsala Sweden

ABSTRACT Karlsson F A Hardell L I and Hellsing K (Departments of Clinical Research Internal Medicine Paediatrics and Clinical Chemistry University Hospital Uppsala Sweden) A prospective study of urinary proteins in early infancy Acta Paediatr Scand 68 663 1979 —Urinary concentrations of protein albumin β_2-microglobulin α amino nitrogen and creatinine were determined in forty-one full term infants on seven occasions up to six months of age Except for β_2-microglobulin the concentrations were highest on the first day followed by a rapid decrease to a constant level within two weeks Protein diminished approximately seven fold albumin twenty fold α amino nitrogen three fold and creatinine five-fold By contrast β_2-microglobulin a low molecular weight protein first increased three fold between day 1 and day 5 thereafter decreasing slowly 17 fold during the first three months of age The data indicate that different kidney functions mature asynchronously

KEY WORDS Albumin β_2-microglobulin α amino nitrogen creatinine urinary concentrations kidney maturation

There is an abundance of quantitative data on protein concentrations in urines from adults and our understanding of the handling of proteins in the mature kidney is steadily growing This knowledge constitutes a necessary basis for the investigation and interpretation of pathological conditions of the kidney In addition analysis of urine may give valuable information not only concerning kidney function but also concerning metabolic and hormonal processes throughout the whole body

Surprisingly little is known about urinary proteins in early life Earlier investigations have demonstrated transitory proteinuria in newborn infants (16 20) however only qualitative measurements were performed Recently we reported urinary concentrations of albumin and β_2-microglobulin in single urine specimens from 138 infants varying in age from full term to one year (9) Protein concentrations were highest in the group 0–1 month of age and decreased rapidly to a constant level after three months Electrophoretic analysis suggests that the proteinuria in early life is of mainly tubular origin

We now report a prospective study of 41 healthy full term infants aimed at characterizing in detail the rapid changes of urinary composition that occur in the neonatal period Further the individual aquisition of adult urinary protein pattern was examined Concentrations of total protein as well as two specific proteins albumin a high molecular weight protein and β_2 microglobulin a low molecular weight protein were determined in urines sampled on seven separate occasions during the first six months of life Also α amino nitrogen was determined as an estimate of urinary output of amino acids and creatinine values measured and evaluated as a possible indicator of diuresis

MATERIAL AND METHODS

Forty-one infants 18 boys and 23 girls born after 38 to 42 weeks of uncomplicated pregnancies were studied The deliveries were normal and all infants were in good health at birth The parents informed consent was obtained Single urine samples were collected within ±10% on the following days 1 5 15 43 90 130 and 180 Some infants did not complete the study or deliver urine on all occasions for various trivial reasons which resulted in a

Table 1 *Urinary concentrations of totalprotein albumin β_2-microglobulin alpha amino nitrogen and creatinine in single urine specimens in early infancy*[a]

Age (days)	n	Totalprotein (mg/l)	Albumin (mg/l)	β_2 microglobulin (mg/l)
1	41	494 (103–2 360)	143 (13 5–1 510)	0 278 (0 021–3 69)
5	40	176 (39 1–798)	20 8 (4 46–96 6)	1 04 (0 067–16 3)
15	29	*55 3 (9 5–324)*	*5 59 (0 96–32 5)*	*0 339 (0 019–6 16)*
43	28	46 4 (11 6–185)	4 35 (1 07–17 7)	0 134 (0 011–161)
90	25	64 4*** (10 1–413)	6 31 (1 56–25 6)	0 061** (0 011–0 331)
130	23	70 0 (10 8–465)	5 78 (1 23–27 1)	0 058 (0 012–0 270)
180	19	65 7 (8 0–538)	4 55 (0 53–39 3)	0 051 (0 009–0 287)

[a] Mean and range (±S D) were calculated from the logarithms of the measured values The mean values for all compounds were tested for differences of statistical significance data for day 1 and day 5 were compared as well as for day 5 and day 90
* $=p<0.05$ ** $=p<0.01$ *** $=p<0.001$

final yield of 71% of the expected urine samples Analysis of a smaller group of 27 infants who completed the sample series with a 92% yield did not result in data (results not shown) that deviated from that of the entire group of 41 infants

Urines were collected and handled as described (9) Albumin was determined by a polymer enhanced immunonephelometric method (11) β_2-microglobulin with the Phadebas β_2 micro test (kindly supplied by Pharmacia Diagnostics Uppsala Sweden) Protein was quantitated by the biuret method after precipitation with phosphotungstic acid (15) using human serum albumin as reference protein α amino nitrogen was estimated as described (10) using glycine as reference Creatinine was determined by an Auto-Analyzer technique (Technicon N 11b)

The urine analyses were evaluated statistically after conversion to logarithmic values Albumin and β_2 microglobulin concentrations in infant urines when plotted on a linear scale are found to be asymmetrically distributed whereas the logarithms of the corresponding data show a more symmetrical distribution suitable for statistical treatment (9) Differences in mean values were analysed with a two tailed t test for paired samples

RESULTS

Table 1 presents the urinary concentrations of protein albumin β microglobulin α amino nitrogen and creatinine determined for the infants during the first six months of life Neonatal proteinuria was regularly found at day 1 *protein* 494 (103–2 362) mg/l (Mean±S D) albumin 143 (13–1 511) mg/l These high concentrations decreased rapidly protein to 176 (39–798) mg/l albumin to 21 (4–97) mg/l on day 5 At 15 days of age a level was reached *that* remained constant throughout the period of the study By contrast the concentrations of β microglobulin showed a different pattern—low levels at day 1, a three fold increase to maximal values at day 5 1 04 (0 07–16 3) mg/l and thereafter a gradual 17 fold decrease over the next three months α amino nitrogen concentrations were highest at birth 172 (75–399) mg α amino N per liter, followed by a three fold decrease to constant values at 15 days of age The data is displayed in Fig 1

The calculated ratios for protein albumin β_2 microglobulin and α amino nitrogen to creatinine are shown in Table 2 The ratios remained constant from 15 days of age except for the β microglobulin/creatinine ratios

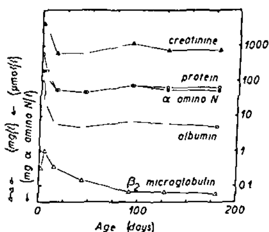


Fig 1 Mean urinary concentration of creatinine protein α amino nitrogen albumin and β_2 microglobulin with increasing age in 41 children studied prospectively Creatinine (µmol/l) α amino nitrogen (mg α amino N/l) protein albumin and β_2 microglobulin (mg/l)

amino N α amino N/l)	Creatinine (µmol/l)
(74 5–399)	4 100 (77?–?1 800)
(36 4–335)	? 500 (46?–13 600)
(19 6–133)	549 (101–? 970)
(16 7–125)	545 (91 6–3 ?50)
(7 6–18?)	1 050 (199–5 550)
(.5 ?–117)	671 (113–3 990)
(13 3–119)	713 (63 9–7 950)

which slowly decreased and leveled off at three months of age Note that the magnitude of the standard deviations calculated for the ratios were not less than those computed for the single values for each compound

Protein excretion by different individuals of the same age varied considerably Five infants had albumin concentrations of more than 600 mg/l in the first day urine the highest being 1318 mg/l β_2 microglobulin concentrations exceeding 4 mg/l were measured in four urines all on day 5 the highest being 12 9 mg/l Subsequent urinary values of albumin or β_2 microglobulin from these children were randomly distributed within the reference range It was not possible in this prospective study based on single urine specimens to distinguish individual infants with consistently high or low protein excretion patterns Further the amounts of urinary proteins showed no relation to the degree of weight loss and weight gain that occurred among the subjects in the neonatal period

DISCUSSION

In studies on urines from adults creatinine ratios can be used to normalize diuresis differences between samples when carefully timed urinary specimens are not available However in the early postnatal period changes in urinary flow and creatinine concentrations are known to occur and hence the use of creatinine as an internal indicator for the diuresis is disputable Thus in a study of seventeen full term infants creatinine levels in serum as well as urine were found to fall about 50% between day 1 and day 6 (18) During this period the glomerular filtration rate increases two fold (6 13 18) and a three to five fold increase in the urinary flow is observed (13 16 21) In the present study a stabilization of urinary creatinine concentrations was noted two weeks after birth However the standard deviations computed for the ratios of the individual substances to creatinine after two weeks of age were larger than those for the substances themselves This suggests that in the neonate/infant other factors besides the diuresis influence creatinine output the urinary concentrations of proteins and the amino acid

Table 2 *Creatinine ratios for totalprotein albumin β_2-microglobulin and alpha amino nitrogen measured in urines of early infancy*

Age (days)	*n*	Totalprotein / creatinine (mg µmol)	Albumin / creatinine (mg µmol)	β microglobulin / creatinine (mg µmol)	alpha amino N / creatinine (mg α amino N µmol ⁻¹)
1	41	0 118 (0 016–0 887)	0 034 (0 003–0 446)	0 067 (0 00?–? 014)	0 041 (0 008–0 ?07)
5	40	0 0?0 (0 01?–0 408)	0 008 (0 00 –0 045)	0 443 (0 016–1 ?94)	0 046 (0 008–0 263)
15	?9	0 101 (0 015–0 679)	0 010 (0 00?–0 057)	0 617 (0 0?0–18 589)	0 091 (0 0?6–0 319)
43	8	0 085 (0 007–1 056)	0 008 (0 001–0 055)	0 ?46 (0 01?–4 805)	0 084 (0 028–0 249)
90	5	0 059 (0 005–0 638)	0 006 (0 001–0 050)	0 053 (0 007–0 365)	0 063 (0 015–0 ?68)
130	23	0 117 (0 011–1 194)	0 009 (0 00 –0 031)	0 091 (0 01 –0 661)	0 082 (0 0?1–0 3?4)
180	19	0 105 (0 007–1 677)	0 007 (0 00?–0 0?4)	0 067 (0 01?–0 373)	0 076 (0 013–0 464)

Mean and range (±S D) were derived from the logarithms of the calculated creatinine ratios

Table 1 Urinary concentrations of totalprotein albumin β₂-microglobulin alpha amino nitrogen and creatinine in single urine specimens in early infancy[a]

Age (days)	*n*	Totalprotein (mg/l)	Albumin (mg/l)	β_2 microglobulin (mg/l)
1	41	494 (103–2 360)	143 (13 5–1 510)	0 278 (0 021–3 69)
5	40	176 (39 1–798)	20 8 (4 46–96 6)	1 04** (0 067–16 3)
15	29	55 3 (9 5–324)	5 59 (0 96–32 5)	0 339 (0 019–6 16)
43	28	46 4 (11 6–185)	4 35 (1 07–17 7)	0 134 (0 011–161)
90	25	64 4 (10 1–413)	6 31 (1 56–25 6)	0 061* * (0 011–0 331)
130	23	70 0 (10 8–465)	5 78 (1 23–27 1)	0 058 (0 012–0 270)
180	*19*	65 7 (8 0–538)	4 55 (0 53–39 3)	0 051 (0 009–0 87)

Mean and range (±S D) were calculated from the logarithms of the measured values The mean values for compounds were tested for differences of statistical significance data for day 1 and day 5 were compared as well for day 5 and day 90

$^{*}=p<0.05$ $^{**}=p<0.01$ $^{*}=p<0.001$

final yield of 71% of the expected urine samples Analysis of a smaller group of 27 infants who completed the sample series with a 92% yield did not result in data (results not shown) that deviated from that of the entire group of *41* infants

Urines were collected and handled as described (9) Albumin was determined by a polymer-enhanced immunonephelometric method (11) β_2-microglobulin with the Phadebas β_2 micro test (kindly supplied by Pharmacia Diagnostics Uppsala Sweden) Protein was quantitated by the biuret method after precipitation with phosphotungstic acid (15) using human serum albumin as reference protein α amino nitrogen was estimated as described (10) using glycine as reference Creatinine was determined by an Auto-Analyzer technique (Technicon N II b)

The urine analyses were evaluated statistically after conversion to logarithmic values Albumin and β_2 microglobulin concentrations in infant urines when plotted on a linear scale are found to be asymmetrically distributed whereas the logarithms of the corresponding data show a more symmetrical distribution suitable for statistical treatment (9) Differences in mean values were analysed with a two-tailed *t* test for paired samples

RESULTS

Table 1 presents the urinary concentrations of protein albumin β microglobulin α amino nitrogen and creatinine determined for the infants during the first six months of life Neonatal proteinuria was regularly found at *day 1* protein 494 (103–2 362) mg/l (Mean±S D) albumin 143 (13–1 511) mg/l These high concentrations decreased rapidly protein to 176 (39–798) mg/l albumin to 21 (4–97) mg/l on day 5 At 15 days of age a level was reached that remained constant throughout the period of the study By contrast the concentrations of β microglobulin showed a different pattern—low levels at day 1, a three fold increase to maximal values at *day 5 1 04 (0 07–16 3) mg/l* and thereafter a gradual 17 fold decrease over the next three months α amino nitrogen concentrations were highest at birth 172 (75–399) mg α amino N per liter followed by a three fold decrease to constant values at 15 days of age The data is displayed in Fig 1

The calculated ratios for protein albumin β microglobulin and α amino nitrogen to creatinine are shown in *Table 2* The ratios remained constant from 15 days of age except for the β microglobulin/creatinine ratios

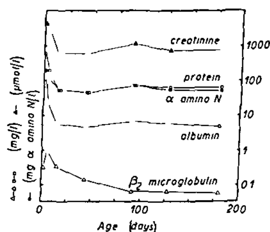


Fig 1 Mean urinary concentration of creatinine protein α amino nitrogen albumin and β_2 microglobulin with increasing age in 41 children studied prospectively Creatinine (μmol/l) α amino nitrogen (mg α amino N/l) protein albumin and β_2 microglobulin (mg/l)

lfa amino N ng α amino N/l)	Creatinine (μmol/l)
77 (74 5–399)	4 100 (77 –71 800)
10* (36 4–335)	7 500* (462–13 600)
1 1 (19 6–133)	549 (101–7 970)
15 7 (16 7–175)	545 (91 6–3 750)
0 9 (77 6–187)	1 050 (199–5 550)
4 7 (5 7–117)	671 (113–3 990)
40 1 (13 3–119)	713 (63 9–7 950)

tion patterns Further the amounts of urinary proteins showed no relation to the degree of weight loss and weight gain that occurred among the subjects in the neonatal period

which slowly decreased and leveled off at three months of age Note that the magnitude of the standard deviations calculated for the ratios were not less than those computed for the single values for each compound

Protein excretion by different individuals of the same age varied considerably Five infants had albumin concentrations of more than 600 mg/l in the first day urine the highest being 1318 mg/l β_2 microglobulin concentrations exceeding 4 mg/l were measured in four urines all on day 5 the highest being 12 9 mg/l Subsequent urinary values of albumin or β_2 microglobulin from these children were randomly distributed within the reference range It was not possible in this prospective study based on single urine specimens to distinguish individual infants with consistently high or low protein excre

DISCUSSION

In studies on urines from adults creatinine ratios can be used to normalize diuresis differences between samples when carefully timed urinary specimens are not available However in the early postnatal period changes in urinary flow and creatinine concentrations are known to occur and hence the use of creatinine as an internal indicator for the diuresis is disputable Thus in a study of seventeen full term infants creatinine levels in serum as well as urine were found to fall about 50% between day 1 and day 6 (18) During this period the glomerular filtration rate increases two fold (6 13 18) and a three to five fold increase in the urinary flow is observed (13 16 21) In the present study a stabilization of urinary creatinine concentrations was noted two weeks after birth However the standard deviations computed for the ratios of the individual substances to creatinine after two weeks of age were larger than those for the substances themselves This suggests that in the neonate/infant other factors besides the diuresis influence creatinine output the urinary concentrations of proteins and the amino acid

Table 2 *Creatinine ratios[a] for totalprotein albumin β₂-microglobulin and alpha amino nitrogen measured in urines of early infancy*

Age (days)	n	Totalprotein / creatinine (mg μmol)	Albumin / creatinine (mg μmol)	β microglobulin / creatinine (mg μmol)	alpha amino N / creatinine (mg α amino N μmol)
3	41	0 118 (0 016–0 887)	0 034 (0 003–0 446)	0 067 (0 002–2 014)	0 041 (0 008–0 207)
5	40	0 070 (0 01 –0 408)	0 008 (0 007–0 045)	0 443 (0 016–17 294)	0 046 (0 008–0 263)
15	29	0 101 (0 015–0 679)	0 010 (0 007–0 057)	0 617 (0 0 0–18 589)	0 091 (0 0 6–0 319)
43	8	0 085 (0 007–1 056)	0 008 (0 001–0 055)	0 746 (0 017–4 805)	0 084 (0 078–0 249)
90	5	0 059 (0 005–0 638)	0 006 (0 001–0 050)	0 053 (0 007–0 365)	0 063 (0 015–0 468)
130	3	0 117 (0 011–1 194)	0 009 (0 00 –0 031)	0 091 (0 01 –0 661)	0 082 (0 021–0 324)
180	19	0 105 (0 007–1 677)	0 007 (0 00 –0 074)	0 067 (0 012–0 373)	0 076 (0 013–0 464)

[a] Mean and range (±S D) were derived from the logarithms of the calculated creatinine ratios

nitrogen. Creatinine ratios thus are of little value in estimating the absolute output of urinary compounds in the case of different single urines.

In our study the concentrations of α-amino nitrogen from day 15 onwards are similar to those reported in earlier papers (3, 17). No data on α-amino nitrogen in immediate post-natal urine has been published to our knowledge. The high values in urines on day 1 and day 5 may reflect incomplete reabsorption of filtered amino acids. Infants are known to excrete about three times as much amino acids as children and adults (2, 3). This is generally thought to reflect a lower tubular reabsorption capacity attributable to the heterogeneity of nephrons in the neonate/infant and resulting in a relative preponderance of glomerular kidney function. The stabilization of urinary α-amino nitrogen concentrations after day 15 suggests that the proximal tubules at this stage have acquired mature capacity with regard to reabsorption of this compound.

Neonatal proteinuria has been described previously in studies using semiquantitative or qualitative acid precipitation techniques. Tausch (20) detected proteinuria in 14.2% of 2 837 catheterized specimens taken immediately after birth. Rhodes et al. (16) tested 61 urines from infants less than 10 days of age and noted proteinuria in 21% of the total material. The frequency of proteinuria fell with increasing age and no full-term infant over three days of age had detectable proteinuria. The rather sparse and somewhat divergent information on the subject in textbooks would indicate that transient proteinuria occurs during the first three days of life (1), trace amounts of protein are seldom present after the fifth day of age (7), proteinuria is frequent during the first ten days of life (12). Another book (19) refers to a study (4) which claims that the presence of urates in urine may give false positive precipitation reactions. Hence it is adduced that the urine of newborns is "free" of protein.

This study extends our earlier quantitative immunological measurements of albumin and β_2-microglobulin in single urine specimens in early infancy (9). Remarkably high protein concentrations were found on day 1 when approximately one third of the total protein could be accounted for by albumin. The high concentrations of total protein fell rapidly with a concomitant marked reduction in the albumin fraction, reaching adult levels at day 15. At this stage qualitative tests for protein in urine from a normal infant should be negative, otherwise special attention is called for.

β_2-microglobulin, a low molecular weight protein (mol. weight 11 800) which is normally reabsorbed in the proximal tubules, is a useful indicator of tubular dysfunction in adults (14). The protein displayed an intriguing pattern—low levels at birth, reaching a peak at day 5 and thereafter declining gradually over the next months. Serum levels of β_2-microglobulin in newborns and infants are slightly elevated compared to adults (5, 8). However, the elevation is not of such a magnitude to cause a tubular load of β_2-microglobulin that could account for the high urinary excretion of the protein. It is more likely that the high concentrations reflect interference with or underdevelopment of tubular capacity to reabsorb the protein. The peak at day 5 and the slow decline to the basal level at three months of age is a pattern markedly different from that of α-amino nitrogen. This suggests that different functions of the proximal tubular epithelium, such as amino acid reabsorption and uptake of low molecular weight proteins, differentiate and mature separately.

The observations reported in this study are thought to reflect dramatic changes in the renal physiology known to occur after birth. However, the mechanisms for the proteinuria are poorly understood and the prerenal, renal, and postrenal contributions to the early neonatal proteinuria is a matter of speculation. When the kidney function of a

newborn is evaluated it should be borne in mind that at two weeks of age proteinuria in quantitative terms should have disappeared One the other hand at this age a high output of β_2 microglobulin and possibly other low molecular weight proteins may be present This is a normal physiological phenomenon that will gradually decrease over the first three months of age

ACKNOWLEDGEMENTS

This work was supported by grants from Kronprinsessan Lovisas fond Dr Michael Johnson kindly assisted with data computations Mrs Majstin Wiik Lundberg and Mr Håkan Engstrom provided skilful technical assistance

REFERENCES

1 Avery G B *Neonatology Pathophysiology and management of the newborns* J B Lippincott Comp Philadelphia 1975 p 493
2 Barlow A & McCauce R A The nitrogen partition in newborn infants urine *Arch Dis Child 23* 2 5 1948
3 Childs B Urinary excretion of free alpha amino nitrogen by normal infants and children *Proc Soc Exp Biol Med 81* 225 195
4 Doxiadis S A Goldfinch M K & Cole N Proteinuria in the newborn *Lancet II* 1242 195
5 Evrin P E & Wibell L The serum levels and urinary excretion of β_2 microglobulin in apparently healthy subjects *Scand J Clin Lab Invest 29* 69 1972
6 Guignard J P Torrado A Da Cunha O & Gautier E Glomerular filtration rate in the first three weeks of life *J Pediatr 87* 268 1975
7 Holt J L McIntosh R & Barnett H L *Pediatrics* Appelton Century-Crofts Inc New York 196 p 150
8 Jonasson L E Evrin P E & Wibell L Content of β_2-microglobulin and albumin in human amniotic fluid *Act Obstet Gynecol Scand 5* 49 1974
9 Karlsson F A & Hellsing K Urinary protein excretion in early infancy *J Pediatr 89* 89 1976
10 Khachadurian A Knox W E & Cullen A M Colorimetric ninhydrin method for total alpha amino acid of urine *J Lab Clin Med 56* 321 1960
11 Lizana J & Hellsing K Polymer enhancement of automated immunological nephelometric analysis as illustrated by determination of urinary albumin *Clin Chem 20* 415 1974
12 Nelson N M Vaughan III V C & McKay R J *Textbook of pediatrics* W B Saunders Comp Philadelphia 1962 p 1180
13 Oh W Oh M A & Lind J Renal function and blood volume in newborn infant related to placental transfusion *Acta Pediatr Scand 56* 197 1966
14 Peterson P A Evrin P E & Berggård I Differentiation of glomerular tubular and normal proteinuria determinations of urinary excretion of β_2 microglobulin albumin and total protein *J Clin Invest 48* 1189 1969
15 Piscator M Proteinuria in chronic cadmium poisoning *Arch Env on Health 5* 325 1962
16 Rhodes P G Hammel C L & Berman L B Urinary constituents of the newborn infant *J Pediatr 60* 18 1962
17 Sereni F McNamara H Shibuya M Kretchmer N & Barnett H L Concentration in plasma and rate of urinary excretion of amino acids in premature infants *Pediatrics 15* 575 1955
18 Sertel H & Scopes J Rates of creatinine clearance in babies less than one week of age *Arch Dis Child 48* 717 1973
19 Smith C A & Nelson N M *The physiology of the newborn infant* C C Thomas Springfield 1976 p 447
20 Tausch M Der Fetalharn *Arch Gynaekol 162* 17 1936
21 Thompson J Observations on the urine of the newborn infant *Arch Dis Child 19* 170 1944

Submitted Nov 9 1978
Accepted March 8 1979

(F A K) Department of Clinical Research and Internal Medicine
University Hospital
S 750 14 Uppsala
Sweden

Acta Pædiatr Scand 68 669–676 1979

BETA 2 MICROGLOBULIN AN INDICATOR OF RENAL TUBULAR MATURATION AND DYSFUNCTION IN THE NEWBORN

A APERIA and U BROBERGER

From the Department of Paediatrics Karolinska Institutet St Goran s Children s Hospital and the Department of Paediatrics and Clinical Chemistry at Karolinska Hospital Stockholm Sweden

ABSTRACT Aperia A & Broberger U (Department of Paediatrics Karolinska Institutet St Goran s Children s Hospital and the Department of Paediatrics and Clinical Chemistry at Karolinska Hospital Stockholm Sweden) Beta 2-microglobulin an indicator of renal tubular maturation and dysfunction in the newborn Acta Paediatr Scand 68 669 1979 —The urinary excretion and proximal tubular reabsorption of beta 2-microglobulin was studied in 17 healthy newborn infants in relation to gestational and post natal age The effect of IRDS and non conjugated hyperbilirubinemia on the tubular reabsorption of the protein was evaluated in 10 IRDS infants and 14 infants with non-conjugated hyperbilirubinemia The urinary excretion of beta 2 microglobulin was determined under standardized conditions When GFR was determined the single injection clearance method was used The filtered load of beta 2 microglobulin was found to increase with increasing gestational age This was due to a rise in plasma beta 2-microglobulin concentration as well as to a rise in the GFR Although the smallest filtered load was recorded in infants with a mean GA of 32 4 weeks these infants had a lower franctional reabsorption of the protein (88 %) than infants with a mean GA of 35 0 weeks or more (98 %) In infants with a GA of 35 weeks or more a glomerulo-tubular balance for beta 2 microglobulin apparently was established In these infants the filtered load of beta 2 microglobulin increased rapidly during the first days of life This was paralleled by an increase in the reabsorptive capacity for the protein In infants with IRDS and in infants with non-conjugated hyperbilirubinemia the fractional reabsorption of beta 2 microglobulin was lower than in control infants of a corresponding gestational and postnatal age This indicates that in the neonatal period the proximal tubular transporting capacity is more vulnerable than the glomerular filtration rate in states of hypoxia and hyperbilirubinemia

KEY WORDS GFR beta 2-microglobulin pre-term and full term infants idiopathic respiratory distress syndrome hyperbilirubinemia

In the human fetus nefronegenesis is completed at about the 36th postmenstrual week The renal functional development is highly dependent on the renal structural development (3 17) It can therefore be expected that infants born before the 36th week in many aspects will differ from full term infants with regard to renal functional maturation Arant has recently shown that the GFR is considerably lower in newborn infants with a GA less than 35 weeks (4) His results also indicate that the glomerulo tubular balance can be different in pre term infants from that in full term infants The fractional reabsorption of glucose and amino acids was found to be relatively lower in infants with a very low gestational age than in full term infants Previous studies have also shown that the excretion of sodium under basal conditions as well as after an oral Na^+ load is higher in pre term than in full term infants (1 2 25)

The renal reabsorption of Na^+ and probably also amino acids are however to a large extent dose dependent and will thus vary not only with GFR but also with serum concentration and the total balance of the respective substance Therefore these substances are not ideal markers of renal tubular maturation

During recent years beta 2 microglobulin

Supported by grants from the Swedish Medical Research Council (B78-19x 3644-06) and Sallskapet Barnavård

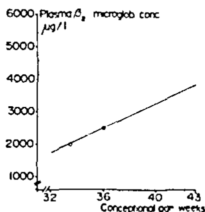


Fig 1 Plasma beta 2 microglobulin concentration in relation to conceptional age in pre term and term infants PNA<4 days (O) PNA>4 days (●)

has gained interest as a marker of renal tubular function (12 24) It is a small naturally occurring protein with a molecular weight of 11 800 that is easily filtered and subsequently reabsorbed in the proximal tubular cells by endocytosis and then transported to the lysosomes where the protein is degraded (19 20) There is no evidence for a transcellular transport of intact protein (5 21) Furthermore no parts of the tubular segments distal to the proximal tubule has been shown to reabsorb any substansial amounts of the protein (18) Normally the serum concentration as well as the fractional reabsorption of beta 2 microglobulin are fairly constant (15 24)

The purpose of this study is to investigate beta 2 microglobulin as a marker of renal tubular maturation in infants born from the 32nd to the 41st gestational week The possible deviation from normal development is evaluated in infants with idiopathic respiratory distress syndrome and in infants with non conjugated hyperbilirubinemia

MATERIALS

This study has been performed on pre term and term infants grouped in the following way

1 *Healthy pre term and term infants* 17 infants with an uncomplicated postnatal course were divided in 3 groups with regard to the gestational age (mean ± 1 S D) Group 1*a* 7 infants with gestational age 32 4±0 5 weeks Group 1*b* 5 infants with gestational age 35 0±0 7 weeks Group 1*c* 5 infants with gestational age 40 8±0 8 weeks

Infants in group 1*a* *b* *c* were studied at the postnatal age of 4–6 days In addition repeated studies with regard to postnatal development has been performed in all infants in group 1*a* at postnatal ages ranging from 1 14 days and in all infants in group 1*b* and *c* at postnatal ages 1–7 days The total serum bilirubin did not exceed 195 µmol/l in any of these infants All pregnancies were uneventful except for pre term deliveries in groups 1*a* and *b* The infants were fed breast milk in amounts of 60–1 0 ml/kg b w /24 hours depending on post natal age

2 *Infants with idiopathic respiratory distress syndrome* 10 infants with a mean gestational age of 34 0 weeks were studied twice first at a mean post natal age of 47 7±16 7 hrs and then at 149 0±29 0 hrs Apgar scores were 7 or more at 1 and 5 min of age except on one case with scores of 3 and 5 respectively The daily fluid requirement was given i v as 10% glucose supplemented with sodium and potassium in amounts of 60 to 170 ml/kg b w /24 hours depending on the post natal age The total serum bilirubin ranged between 235 and 318 µmol/l This group of infants has been described in detail in a previous study (7)

3 *Infants with hyperbilirubinemia* (*a*) 8 pre term infants with mean gestational age 35 0 weeks were studied at a mean post natal age of 114 1±18 9 hrs Their total serum bilirubin ranged between 255 and 376 µmol/l (*b*) 6 term infants with mean gestational age 39 2 weeks were studied at a mean post natal age of 125 7±27 3 hrs The total serum bilirubin ranged between 291 and 390 µmol/l

All pregnancies were uneventful except for the pre term deliveries Apgar scores were 7 or more at 1 min and 8 or more at 5 min of age All infants were fed breast milk in amounts of 60 to 120 ml/kg b w /24 hrs depending on post natal age

The weight and length of all infants corresponded to the Swedish standards (11) The pre term infants were kept in *Isolette incubators* *Informed parental consent* was obtained in all cases studied The protocol was approved by the Committee of Ethics at Karolinska sjukhuset

METHODS

The urinary excretion of beta 2 microglobulin was studied during standardized fluid expansion Breast milk diluted with water (1 1) was administered by gastric tube in an amount corresponding to 2% of the body weight during one hour and then in an amount of 0 5% every thirty minutes Infants with IRDS were given 5 5% glucose i v corresponding to 1% of the body weight during the first hour This was followed by 0 5% of the body weight per hour When a stable diuresis was established the collection of urine started The urine was collected after spontaneous voiding in urinary bags for a time period of 4 to 6 hours Every portion of urine was removed and kept in the refrigerator during completion of the sampling period The urine was then frozen and stored until analyzed The beta 2 microglobulin excretion was calculated and expressed as the average hourly excretion per 1 73 m² B S

The beta 2 microglobulin concentration in urine and serum was determined with the Phadeba β_2 micro test (Pharmacia Diagnostics Uppsala Sweden)

Table I *Urinary excretion of β_2-microglobulin in healthy 4–6 days old pre term and term infants*

Values are mean ± S D

Gestational age weeks	32 4±0 5	35 0±0 7	40 8±0 8
Excreted β_2-micro µg/1 73 m B S /min	5 6±? 2	1 3±0 6	3 5±? 3
Hematocrit %	57 7±6 8	53 0±4 6	48 0±3 8
n	7	5	5

The tubular reabsorption of beta 2 microglobulin was calculated as the difference between filtered and excreted beta 2 microglobulin in urine

GFR was determined with the single injection inulin clearance method (6) For calculation of the filtered amount of beta 2 microglobulin in groups 1*a*–*b* and 3*a*–*b* previously published GFR values of comparable groups were used (2 7 8)

Hematocrit was estimated in glass capillaries rinsed with heparin and centrifuged for 5 min at 12 000 × *g*

The total serum bilirubin concentration was determined in duplicate with a bilirubinometer (American Optical Corp)

Conjugated bilirubin was determined with Nosslin s method (?3)

Gestational age was determined with the method described by Dubowitz et al (10)

Statistical calculations included the Student s *t* test and linear regression analysis

The following abbreviations have been used IRDS= idiopathic respiratory distress syndrome GA=gestational age GFR=glomerular filtration rate PNA=post natal age

RESULTS

Pre term and term control infants

The plasma concentration of beta 2 microglobulin increased with increasing conceptional age in control infants as is shown in Fig 1 The positive correlation between plasma concentration and conceptional age is significant (0 0025>*p*>0 005) *r*=0 64

Urinary beta 2 microglobulin excretion in relation to gestational age in 4–6 days old infants The results obtained from the studies at a postnatal age of 4–6 days in infants with a mean of GA of 32 4 35 0 and 40 8 weeks are shown in Table 1 The urinary excretion of beta 2 microglobulin was higher (0 0025>*p*>0 005) in infants with a mean of GA of 32 4 weeks than in infants with a mean of 35 0 weeks who had the lowest observed urinary excretion of beta 2 microglobulin From a mean GA of 35 0 to 40 8 weeks the urinary excretion of beta 2 microglobulin again increased and this increase was significant (0 05>*p*>0 025) The urinary excretion of beta 2 microglobulin was not significantly different in infants with a mean GA of 32 4 weeks and in infants with a mean GA of 40 8 weeks

Among the patients included in Table 1 the GFR was only determined in the group of infants with mean GA 40 8 weeks In previous studies performed under similar conditions in this laboratory the GFR averaged 22 0±6 7 ml/1 73 m² B S /min in 4–6 days old infants with a mean GA of 32 0 weeks (2) and 38 3± 8 1 ml/1 73 m² B S /min in 4–6 days old infants with a mean GA of 34 4 weeks (7) These values were used to calculate the average amount of filtered and reabsorbed beta 2 microglobulin in the two groups of pre term infants In the full term infants the amount of filtered and reabsorbed beta 2 microglobulin could be cal

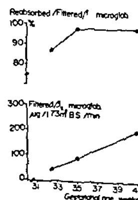


Fig 2 Fractionally reabsorbed and filtered beta 2 microglobulin in relation to gestational age in healthy 4–6 days old pre term and term infants

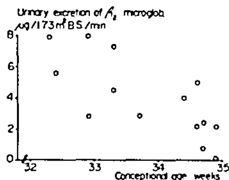


Fig. 3. Urinary excretion of beta 2 microglobulin in relation to conceptional age in pre term infants with GA<35 weeks.

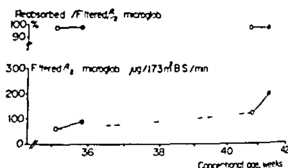


Fig. 4. Fractionally reabsorbed and filtered beta 2 microglobulin in relation to conceptional age in pre term and term infants at PNA 1–2 days (O) and 4–6 days (●).

culated in each individual patient. Changes in the amount of filtered and reabsorbed beta 2 microglobulin with increasing GA are shown in Fig. 2. There is a continuous rise in the amount of filtered beta 2 microglobulin with increasing GA. The amount of filtered beta 2 microglobulin is even when related to body surface area 5 fold higher in the infants with a GA of 40.8 weeks than in the infants with a GA of 32.4 weeks. Yet the fractional reabsorption of beta 2 microglobulin is less complete (87.2%) in the infants with a GA of 32.4 weeks than in the infants with a GA of 40.8 weeks (98.0%). Already at the gestational age of 35 weeks the fractional reabsorption of beta 2-microglobulin appears to have reached a steady level at around 98%.

Urinary beta 2 microglobulin excretion in relation to post natal age in pre term and full term control infants. The post natal development of the renal handling of beta 2 microglobulin is illustrated in Figs. 3 and 4 and Table 2. Repeated studies in infants with a GA<35 weeks showed that there is an indirect relationship between the urinary beta 2 microglobulin excretion and the conceptional age (see Fig. 3) when the latter ranges from 32.5–35 weeks. $r=-0.75$ ($0.0025>p>0.005$). In infants with a mean GA of 35.0 and 40.8 weeks the studies were performed twice, at the post natal age of 1–2 days and 4–6 days. In both the pre term and term infants there was an increase in the average excretion of beta 2 microglobulin with increasing PNA (Table 2). The increase in urinary excretion paralleled an increase in filtered load and the fractional reabsorption of beta 2 microglobulin remained constant (Fig. 4).

The relationship between filtered and reabsorbed beta 2 microglobulin. The possibility of a saturated transport capacity for beta 2 microglobulin was examined in Fig. 5. All individual values obtained from fullterm infants aged 1–5 days are included. Since there is an increase in the GFR from 1–2 days of age (34.2±7.1 ml/1.73 m² B.S./min) to 4–6 days of

Table 2. *Postnatal changes of the urinary β_2-microglobulin excretion in pre term and term healthy infants*

Values are mean ± S.D.

	GA 35 weeks		GA 40 weeks	
Post natal age, hrs	28.8±2.8	136.6±22.0	37.6±11.8	103.8±11.7
Excreted β_2 micro µg/1.73 m² B.S./min	1.0± 0.9	1.3± 0.6	1.5± 1.3	3.5± 2.3
Hematocrit %	53.6± 4.0	53.0± 4.6	48.8± 4.8	48.0± 3.8
n	5	5	5	5

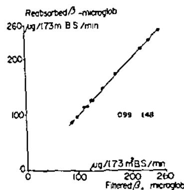


Fig. 5. Relation between filtered and reabsorbed beta 2 microglobulin. Individual values in term infants with a PNA of 1–5 days (●). Solid line represents regression line for term infants. Mean values for pre term infants (GA 35w) at a PNA of 1–2 and 4–6 days (O).

age (53.2±11.1 ml/B.S./min) the filtered loads will range from 99–253 µg/1.73 m² B.S./min. In the observed range of loads there is a straight line relationship between filtered and reabsorbed beta 2 microglobulin. The calculated values for the infants with a mean GA of 35.0 weeks aged 1–2 days and aged 4–6 days will fall on the extension of this line. Thus the increase in filtered load of beta 2 microglobulin depending on increasing GA and PNA in infants with a GA≥35 weeks will not saturate the capacity to reabsorb beta 2 microglobulin.

Infants with IRDS

The plasma concentration of beta 2 microglobulin in IRDS infants (4825±1696 µg/l) was significantly higher than in pre term controls (2195±479 µg/l) of a similar GA and PNA ($p<0.005$). The renal handling of beta 2 microglobulin in IRDS infants is shown in Table 3. IRDS infants with a mean GA of 34.0 weeks excreted more beta 2 microglobulin in urine than control infants with a mean GA of 35.0 weeks ($0.025>p>0.125$). The relationship between filtered and reabsorbed beta 2 microglobulin was examined in Fig. 6. Since the studies in control infants indicated a development of the reabsorptive capacity for beta 2 microglobulin until the 35th week of gestation IRDS infants with conceptional age ≥35 weeks were compared to the control infants. It was found that IRDS infants consistently reabsorbed less beta 2 microglobulin than control infants at a corresponding tubular load.

Infants with hyperbilirubinemia

The plasma concentration of beta 2 microglobulin was determined in three pre term and three term infants with non conjugated hyperbilirubinemia. The plasma concentration was higher in pre term hyperbilirubinemic infants 4997±303 µg/l than in the controls with a cor

Table 3. *Filtered amount, urinary excretion and reabsorption of β_2-microglobulin in IRDS infants*

Values are mean ± S.D.

	GA 34.0 weeks	
Post natal age, hrs	4 ?±16.7	149.0± 9.0
Filtered β_2-microglobulin µg/1.73 m² B.S./min	195.1±59.7	133.8±4? 9
Urinary excretion of β_2-microglobulin µg/1.73 m² B.S./min	9.7± 7.8	8.3± 6.7
Reabsorbed β_2-micro µg/1.73 m² B.S./min	185.3±60.7	1?5.5±43.3
Reabsorption, %	94.6± 4.6	93.?± 5.1
Hematocrit, %	47.3± 5.6	4 .8± 6.5
n	10	10

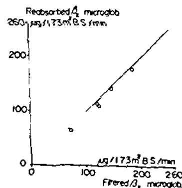


Fig. 6. Relation between filtered and reabsorbed beta 2 microglobulin in IRDS infants of conceptional age ≥35 weeks (O). Solid line represents regression line for term infants.

Table 4 *Urinary excretion of β₂-microglobulin in pre term and term infants with hyperbilirubinemia*

Values are mean ± S D

Gestational age weeks	35 0± 0 8	39 2± 1 2
Post natal age hrs	114 1±18 9	125 7±27 3
Excreted β_2 micro $\mu g/1\ 73\ m^2$ B S /min	6 3± 4 9	6 9± 6 8
Hematocrit %	54 3± 6 8	52 5± 4 5
n	8	6

responding gestational age 2 195±978 μg/l ($p<0\ 005$) but was about the same in fullterm hyperbilirubinemic infants 4 142±1 260 μg/l as in full term control infants 3 817±619 μg/l The urinary excretion of beta 2 microglobulin (Table 4) was higher in pre term and term infants with hyperbilirubinemia than in the corresponding controls although the difference was significant only for pre term infants ($0\ 025>p>0\ 0125$) Individual determinations of glomerular filtration rates were obtained only in a few of the hyperbilirubinemic infants in this study For this reason the average amount of filtered and reabsorbed beta 2 microglobulin was calculated using values obtained previously for GFR in pre term hyperbilirubinemic infants (31 1±6 3 ml/1 73 m² B S /min) and full term hyperbilirubinemic infants (36 0±7 3 ml/1 73 m /B S /min) (8) The average amount of reabsorbed beta 2 microglobulin when related to the filtered load of beta 2 microglobulin (Fig 7) was lower in pre term and term hyperbilirubinemic infants than in control infants

DISCUSSION

Previous knowledge of the renal handling of beta 2 microglobulin has mainly been derived from determination of beta 2 microglobulin in amniotic fluid (14 16) Apparently the amniotic content of beta 2 microglobulin is determined by the fetus rather than by the mother The concentration of beta 2 microglobulin in amniotic fluid increases until the 24th week of pregnancy Thereafter a fall in the concentration occurs which appears to be completed at the 35th week of pregnancy Most likely these changes are due to the combined effect of a developmental increase in production and developmental changes in the renal handling of beta 2 microglobulin This study has shown that there is a continuous rise in plasma beta 2 microglobulin concentration from at least the 32nd to the 42nd conceptional week which in part must reflect an increased production of the protein with growth of the fetus and the infant The continuous fall in the urinary beta 2 microglobulin excretion that occurred from the 32nd to the 35th postmenstrual week could explain the fall in amniotic concentration of beta 2 microglobulin (14 16)

Although the infants with GA below 35 weeks had the lowest filtered load of beta 2 microglobulin the tubular reabsorption of this substance was less complete than in infants with a higher GA This suggests rapid developmental changes of the proximal tubular cells from the 32nd to the 35th postmenstrual week It has previously been shown (4) that the fractional excretion of glucose and of amino acids fall abruptly from the 28th to the 30th postmenstrual week and then continue to fall but more slowly until about the 36th postmenstrual week In full term infants the glomerulo-

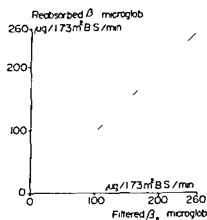


Fig 7 Relation between average values for filtered and reabsorbed beta 2 microglobulin in pre term (O) and term (●) infants with hyperbilirubinemia Solid line represents regression line for control infants

tubular balance for glucose is about the same as in adults (9) The fractional excretion of sodium is also relatively high in infants with gestational age below 36 weeks (2 25) Beta 2 microglobulin glucose and amino acids and Na are completely or to a large extent reabsorbed in the proximal tubule but with different transport pathways (20 26 27) It could therefore be concluded that the maturation of the renal proximal tubular cells will in most aspects be accelerated in the middle of the last trimester This is well in agreement with previous experimental studies of the guinea pig fetus (22)

The tubular load of beta 2 microglobulin increased both with increasing gestational and post natal age The increase was due to a rise in plasma beta 2 microglobulin concentration as well as to a rise in glomerular filtration rate When the filtered load increased in infants with gestational age above 35 weeks the glomerulo-tubular balance for beta 2 microglobulin was well maintained Thus the tubular capacity to reabsorb the protein is rapidly adapted to the demands i e the filtered load in infants with gestational age above 35 weeks In IRDS infants the filtered load of beta 2 microglobulin was higher than in control infants of corresponding GA and PNA but in the same range as in control infants of higher GA but of the same PNA The fractional reabsorption of beta 2 microglobulin in IRDS infants was lower than in control full term infants and appeared to be lower than in control infants with conceptional age 35 weeks The relatively low fractional reabsorption of beta 2 microglobulin in IRDS infants can then be due either to a saturation of the transport capacity for beta 2 microglobulin at the 35th–36th post menstrual week or by an hypoxic effect on the proximal tubular epithelium Previous studies on renal function in IRDS have shown that the GFR can be depressed as a function of hypoxia (13) but that there appears to be a threshold for this effect Thus infants with a moderate degree of IRDS can have a normal or even a supernormal GFR (7) It should be noted however that the fractional excretion of sodium in those infants was higher than in control infants There are thus several indirect data that suggest that tubular function is more vulnerable than glomerular function in hypoxic infants

In hyperbilirubinemia the fractional reabsorption of beta 2 microglobulin appeared to be lower both in pre term and in term infants than it was in control infants of corresponding gestational and post natal age In full term hyperbilirubinemic infants the filtered load of beta 2 microglobulin was not larger than the filtered load in the full term control infants The relatively low fractional reabsorption of beta 2 microglobulin in the term hyperbilirubinemic infants can therefore only be attributed to an effect of hyperbilirubinemia on the proximal transport mechanism A relatively high fractional excretion of sodium has also previously been shown in hyperbilirubinemia (8) This indicates that this condition in infants will also result in a relatively unspecific effect on the proximal tubular epithelium

The results from this study have thus shown that glomerular functional development precedes tubular functional development until about the 34th postmenstrual week There after glomerulo-tubular balance appears to be attained at least for beta 2 microglobulin that is exclusively reabsorbed in the proximal tubule by only one transport mechanism Even after the 34th postmenstrual week the tubular transporting capacity does however appear to be more vulnerable than the glomerular filtration rate in states of disease where the general condition of the infant is disturbed

REFERENCES

1 Aperia A Broberger O Thodenius K & Zetterström R Renal response to an oral sodium load in newborn full term infants *Acta Paediat Scand 61* 670 197?

2 Aperia A Broberger O Thodenius K & Zetterström R Developmental study of the renal response to an oral salt load in pre term infants *Acta Paediatr Scand 63* 517 1974

3 Aperia A & Larsson L Correlation between fluid

reabsorption and proximal tubule ultrastructure during development of the rat kidney *Acta Physiol Scand 105* 11 1979
4 Arant B S Developmental patterns of renal functional maturation compared in the human neonate *J Pediatr 92* 705 1978
5 Bode F Ottosen P D Madsen K & Maunsbach A B Does transtubular transport of protein occur in the proximal tubule? *Proc Int Symp Correlation of renal ultrastructure and function* Univ Aarhus Denmark 1978
6 Broberger U Determination of glomerular filtration rate in the newborn *Acta Paediatr Scand 62* 625 1973
7 Broberger U & Aperia A Renal function in idiopathic respiratory distress syndrome *Acta Paediatr Scand 67* 313 1978
8 Broberger U & Aperia A Renal function in infants with hyperbilirubinemia *Acta Paediatr Scand 68* 1979
9 Brodehl J Franken A & Gellissen K Maximal tubular reabsorption of glucose in infants and children *Acta Paediatr Scand 61* 413 1972
10 Dubowitz L M S Dubowitz V & Goldberg C Clinical assessment of gestational age in the newborn infant *J Pediatr 77* 1 1970
11 Engstrom I Karlberg P Olsson T & Selstam U 1971 Copyright
12 Fredriksson Å Renal handling of β_2 microglobulin in experimental renal disease *Scand J Clin Lab Invest 35* 591 1975
13 Guignard J P Torrado A Mazouni S M & Gautier E Renal function in respiratory distress syndrome *J Pediatr 88* 845 1976
14 Hall P W & Roux J F Amniotic fluid beta 2 microglobulin concentration—an index of gestational age *Am J Obstet Gynecol 120* 56 1974
15 Johansson B G & Ravnskov U The serum level and urinary excretion of α microglobulin β_2 microglobulin and lysosyme in renal disease *Scand J Urol Nephrol 6* 249 1972
16 Jonasson L E Evrin P E & Wibell L Content of β_2 microglobulin and albumin in human amniotic fluid *Acta Obstet Gynecol Scand 53* 49 1974
17 Larsson L & Horster M Ultrastructure and fluid transport in isolated perfused developing proximal tubules *J Ultrastruct Res 54* 276 1976
18 Mausbach A B Functions of Lysosomes in Biology and Pathology *In* J T Dingle & H B Fell (eds) *Lysosomes in biology and pathology* North Holland Publ Comp Amsterdam and London 1969 vol p 115
19 Maunsbach A B Ultrastructure of the proximal tubule *In* S R Geiger (ed) *Handbook of physiology* Waverly Press Inc Baltimore Maryland 19 section 8 p 31
20 Maunsbach A B & Christensen E I Lysosomal accumulation and digestion of β_2-microglobulin in kidney tubules *IRCS* 2 1737 1974
21 Maunsbach A B & Christensen E I Uptake and digestion of low molecular weight protein in rat kidney tubules *Proc 6th Int Congr Nephrol* Karger Basel 1976 p 387
22 Merlet Bernichou C Muffat Joly M & de Rouffignac C Renal transport of glucose and phosphate in the fetal and newborn guinea pig *Proc Int Union Physiol Sci vol 13* Paris 1977 p 503
23 Nosslin B The direct diazo reaction of bile pigment in serum *Scand J Clin Lab Invest 12* Suppl 49 p 1 1960
24 Peterson P A Evrin P E & Berggård I Differentiation of glomerular tubular and normal proteinuria *J Clin Invest 48* 1189 1969
25 Siegel S R & Oh W Renal function as a marker of human fetal maturation *Acta Paediatr Scand 65* 481 1976
26 Ullrich K J Renal tubular mechanism of organic solute transport *Kidney Int 9* 134 1976
27 Windhager E E & Giebisch G Proximal sodium and fluid transport *Kidney Int 9* 121 1976

Submitted Dec 6 1978
Accepted Febr 21 1979

(A A) Department of Paediatrics
St Goran s Children s Hospital
Box 12500
S 11281 Stockholm
Sweden

Acta Pædiatr Scand 68 677–681 1979

THE EXCRETION OF C_6–C_{10} DICARBOXYLIC ACIDS IN THE URINE OF NEWBORN INFANTS DURING STARVATION

Evidence for ω-oxidation of Fatty Acids in the Newborn

N GREGERSEN and J INGERSLEV

From the Research Laboratory for Metabolic Disorders University Department of Clinical Chemistry and the University Department of Obstetrics and Gynaecology Århus Kommunehospital Århus Denmark

ABSTRACT Gregersen N and Ingerslev J (Research Laboratory for Metabolic Disorders University Department of Clinical Chemistry and University Department of Obstetrics and Gynaecology Aarhus kommunehospital Aarhus Denmark) The excretion of C_6–C_{10}-dicarboxylic acids in the urine of newborn infants during starvation Acta Paediatr Scand 68 677 1979 —The excretion of C_6–C_{10}-dicarboxylic acids i e adipic suberic and sebacic acids was measured during the three first days of life in 3 fasting newborns 2 newborns fed with isocaloric glucose and 2 newborns given mothers milk On the second and third day of life the starved children excreted 27–84 mmol adipic acid/mol creatinine 6–22 mmol suberic acid/mol creatinine and 4–7 mmol sebacic acid/mol creatinine The excretion of C_6–C_{10}-dicarboxylic acids in the neonates given glucose or mothers milk was for the first three days of life 0–9 mmol adipic acid/mol creatinine 0–10 mmol suberic acid/mol creatinine and 0–4 mmol sebacic acid/mol creatinine The latter amounts are equivalent to the excretion of dicarboxylic acids in older children It is argued that the detected dicarboxylic acids are formed by ω-oxidation of long-chain monocarboxylic acids followed by β-oxidation and that the excreted amounts reflect ω-oxidation activity It is speculated that the substantial ω-oxidation activity in the starving newborn serve to provide succinyl-CoA substrate for the citric acid cycle and for gluconeogenesis

KEY WORDS Neonatal metabolism C_6–C_{10}-dicarboxylic acids ω-oxidation of fatty acids

Recently we reported the urinary excretion of a number of low molecular weight organic acids of the newborn infant (5) The excretion of adipic acid ranged from 0–59 mmol/mol creatinine on the first day of life and from 0–46 mmol/mol creatinine on the fourth day The median excretion was approximately 14 mmol/mol creatinine which is much higher than the normal adult excretion of 2–7 mmol/mol creatinine (95% reference interval) Preliminary investigations in this laboratory showed that the excretion of other medium-chain dicarboxylic acids such as suberic and sebacic acids is also higher in neonates than in older children and adults These dicarboxylic acids are most probably biosynthesized by ω-oxidation of long-chain monocarboxylic acids followed by β-oxidation through the ordinary fatty acid oxidation pathway (12 13) In order to study the possible existence of biologically significant ω-oxidation in the neonate we measured the excretion of adipic suberic and sebacic acids in the urine of newborn infants fed with either mothers milk isocaloric glucose or electrolytes in water (starvation)

MATERIALS AND METHODS

Clinical material

Urine was collected from 7 healthy full term neonates (2 females and 5 males) randomized to three groups Each group represents a particular three days feeding program

1 Sterile aqueous solution of 13 6 mmol/l potassium chloride and 5 mmol/l sodium chloride (3 neonates)

2 Sterile aqueous solution of glucose 181 3 g/l and electrolytes as in group 1 (2 neonates)

3 Breast milk from the hospital milk bank (2 neonates)

The solutions for groups 1 and 2 were prepared at the hospital pharmacy All neonates were given 7 feedings per day starting 2 hours after delivery with 15–30 ml portions on day one On days 2 and 3 the meals were increased

to 40 and 50 ml respectively Aspiration of the stomach content was performed prior to feeding Typically the aspirate amounted to 4–8 ml In no instance did the 3 newborns in feeding group 1 reveal any clinical sign of hypoglycemia No significant difference in mean weight loss was recorded between the groups The neonates studied were up for adoption and were treated and observed in the prematurity ward Informed consent was given by the head of the department

Two reference groups were incorporated in the study 1) 10 normal newborn at day 1–4 of life (For details of feeding program see ref 5) 2) 10 normal children 2–9 years of age given a mixed Danish diet

Chemicals

DL 3 OH butyric acid (Na salt) was obtained from Merck A G (Darmstadt Germany) Adipic suberic sebacic and diethylglutaric acids were purchased from Koch Light Laboratories Ltd (Bucks England)

Stationary phase for gas chromatography Dexsil 300 was obtained from Analabs Inc (North Haven USA) and column support chromosorb W (HP) from Koch Light Laboratories Ltd Bis (trimethylsilyl) trifluoroacetamide (BSTFA) containing 1% Trimethylchlorosilane (TMCS) was purchased from Pierce Chemical Co (Rockford Ill USA)

Analytical procedure

The extraction procedure was as reported previously (5) except that the extracted acids were silylated with 200 μl of BSTFA/1% TMCS and heated at 60°C for 30 min before the analysis The metabolic profiles were obtained by means of a Hewlett Packard 5830 gas chromatograph (5) The amounts of 3 OH butyric acid were determined from the metabolic profiles with diethylglutaric acid as internal standard No correction for detector response factors was made Adipic suberic and sebacic acids were measured with diethylglutaric acid as internal standard mass fragmentographically by an AEI (KRATOS) MS 30 mass spectrometer supplemented with a 6-channel selected ion detector (SID) unit For all the compounds including the internal standard M 15 was used as the ion to be monitored The standard curves were linear from 0–55 mmol/mol creatinine and the within-day imprecision was <10% for the three dicarboxylic acids Details of the analysis will be reported elsewhere

RESULTS

A gas chromatogram of a urinary metabolic profile of organic acids from a starved neonate is demonstrated in Fig 1 The profiles of the investigated children were recorded in order to detect any gross difference in the excretion pattern in the three feeding groups The only significant difference was the excretion of 3 OH butyric acid and the dicarboxylic acids

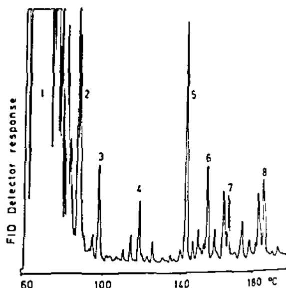


Fig 1 Flame ionisation detector (FID) response of a silylated metabolic profile of organic acids from a starved infant the second day of life The peaks represent 1) solvent 2) lactic acid 3) 3 OH butyric acid 4) succinic acid 5) adipic acid 6) diethylglutaric acid (internal standard) 7) suberic acid and 8) sebacic acid+hippuric acid (gas chromatographic conditions see ref (5))

The concentration of 3 OH butyric acid was determined directly from the profiles while the dicarboxylic acids were quantitated by means of the more sensitive and specific mass fragmentographic method A mass fragmentographic profile of the dicarboxylic acids is shown in Fig 2 The data obtained are listed in Table 1 The numbers are too few for statistical evaluation In the present study the excretion pattern of the infants fed with mothers milk or glucose are comparable to that of older children (Table 1) From the second day on the excretion of adipic acid and suberic acid is clearly much higher in the starved than in the fed infants

DISCUSSION

During the first days of life until breast feeding is sufficiently instituted the normal newborn infant is in a state of starvation and depends almost entirely on endogenous fat for energy requirements (8 14) In the present study we investigated the particular metabolic situation of absolute starvation in the neonatal

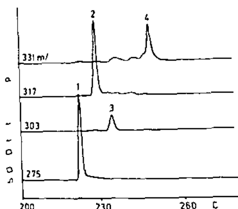


Fig 2 Selected ion detection (SID) responses of the profiles of ion fragments m/e 275 303 and 331 in the silylated extract from the same urine as that in Fig 1 The peaks represent 1) adipic acid 2) diethylglutaric acid (internal standard) 3) suberic acid and 4) sebacic acid The gas chromatographic column was a 180 cm×3 mm (i d) glass coil packed with 3% Dexsil 300 on Chromosorb W (HP) The column temperature was programmed from 200°C at 15 C/min to 300°C The helium carrier flowrate was 30 ml/min The injection port and the glass sample line including the jet separator between the gas chromatograph and the mass spectrometer were 250°C and the ion source temperature was 175 C Ionizing and accelerating potentials were 70 eV and 4 kV respectively

period in one group of infants while we prevented the starvation in two other groups fed with either sufficient amounts of glucose or mothers milk to cope with energy demands The excretion of 3 OH butyric acid increasing to moderate levels in the fasting groups serve to characterise the state of excessive lipid catabolism This metabolic situation is also reflected in the urinary excretion of substantial amounts of C_6–C_{10} dicarboxylic acids in the starved infants compared to the low excretion of these acids in the fed newborns

In light of the investigations of Pettersen (10 11 12 13) and Bjorkhem (1 2) it is most probable that the source of urinary dicarboxylic acids in starvation are fatty acids ω-oxidised to long-chain dicarboxylic acids followed by β-oxidation The increased ω-oxidation in liver homogenates from starved and diabetic rats (1) indicates a positive correlation between the amounts of dicarboxylic acids excreted and ω oxidation in the intact organism Therefore a substantial excretion of dicarboxylic acids in the starved group most probably reflects the degree of ω-oxidation On the other hand in the groups fed with carbohydrate or milk the ω-oxidation was comparable to that found in older non fasting children The finding of increased ω-oxidation in starved infants is in accordance with Bjorkhem who demonstrated an inverse relationship between ω-oxidation and glyceride biosynthesis (2) which is low during starvation (7) The relative metabolic importance of ω oxidation compared to the ordinary β oxidation cannot be determined from the presented results as it is probable that the excreted amounts of dicarboxylic acids represents an overflow of ω oxidised fatty acids The present results however and those of Bjorkhem (1 2) and Pettersen (10 11) very strongly indicate that ω-oxidation is more important in states of excessive fat catabolism i e starvation and diabetes than in the normal state

Wada & Usami (16) estimated that about 15% of palmitic acid was subjected to ω oxidation followed by β-oxidation in starved diabetic rats Hemmelgarn and coworkers estimate an initial ω-oxidation percentage of 40 in non ketotic diabetic rats whereas the remaining fatty acid decomposition is due to primary β-oxidation (4) These figures suggest that ω oxidation plays a significant biological role The excreted dicarboxylic acids must have been derived from their CoA-derivatives If adipyl CoA is β-oxidised succinyl CoA will be formed Succinyl CoA may serve as substrate for the citric acid cycle and consequently as a gluconeogenetic precursor The finding of Wada & Usami (16) corroborate this hypothesis After administration of dicarboxylic acids to starved rats a decrease in blood ketone bodies was detected and a higher incorporation of ^{14}C into blood glucose from 1 ^{14}C-dicarboxylic acids than from 1 ^{14}C monocarboxylic acids was recorded In the newborn the biological consequence of ω-oxidation of fatty acids resulting in the formation

to 40 and 50 ml respectively Aspiration of the stomach content was performed prior to feeding Typically the aspirate amounted to 4–8 ml In no instance did the 3 newborns in feeding group 1 reveal any clinical sign of hypoglycemia No significant difference in mean weight loss was recorded between the groups The neonates stud ied were up for adoption and were treated and observed in the prematurity ward Informed consent was given by the head of the department

Two reference groups were incorporated in the study 1) 10 normal newborn at day 1–4 of life (For details of feeding program see ref 5) 2) 10 normal children 2–9 years of age given a mixed Danish diet

Chemicals

DL 3-OH butyric acid (Na salt) was obtained from Merck A G (Darmstadt Germany) Adipic suberic sebacic and diethylglutaric acids were purchased from Koch Light Laboratories Ltd (Bucks England)

Stationary phase for gas chromatography Dexsil 300 was obtained from Analabs Inc (North Haven USA) and column support chromosorb W (HP) from Koch Light Laboratories Ltd Bis (trimethylsilyl) trifluoroacet amide (BSTFA) containing 1% Trimethylchlorosilane (TMCS) was purchased from Pierce Chemical Co (Rock ford Ill USA)

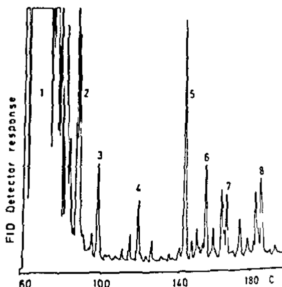


Fig 1 Flame ionisation detector (FID) response of a silylated metabolic profile of organic acids from a starved infant the second day of life The peaks represent 1) solvent 2) lactic acid 3) 3 OH butyric acid 4) succinic acid 5) adipic acid 6) diethylglutaric acid (internal standard) 7) suberic acid and 8) sebacic acid+hippuric acid (gas chromatographic conditions see ref (5))

Analytical procedure

The extraction procedure was as reported previously (5) except that the extracted acids were silylated with 200 µl of BSTFA/1% TMCS and heated at 60°C for 30 min be fore the analysis The metabolic profiles were obtained by means of a Hewlett Packard 5830 gas chromatograph (5) The amounts of 3 OH butyric acid were determined from the metabolic profiles with diethylglutaric acid as internal standard No correction for detector response factors was made Adipic suberic and sebacic acids were measured with diethylglutaric acid as internal standard mass frag mentographically by an AEI (KRATOS) MS 30 mass spectrometer supplemented with a 6 channel selected ion detector (SID) unit For all the compounds including the internal standard M 15 was used as the ion to be moni tored The standard curves were linear from 0–55 mmol/mol creatinine and the within day imprecision was <10% for the three dicarboxylic acids Details of the analysis will be reported elsewhere

RESULTS

A gas chromatogram of a urinary metabolic profile of organic acids from a starved neonate is demonstrated in Fig 1 The profiles of the investigated children were recorded in order to detect any gross difference in the excretion pattern in the three feeding groups The only significant difference was the excretion of 3 OH butyric acid and the dicarboxylic acids

The concentration of 3 OH butyric acid was determined directly from the profiles while the dicarboxylic acids were quantitated by means of the more sensitive and specific mass fragmentographic method A mass fragmento graphic profile of the dicarboxylic acids is shown in Fig 2 The data obtained are listed in Table 1 The numbers are too few for sta tistical evaluation In the present study the excretion pattern of the infants fed with mothers milk or glucose are comparable to that of older children (Table 1) From the sec ond day on the excretion of adipic acid and suberic acid is clearly much higher in the starved than in the fed infants

DISCUSSION

During the first days of life until breast feed ing is sufficiently instituted the normal new born infant is in a state of starvation and depends almost entirely on endogenous fat for energy requirements (8 14) In the present study we investigated the particular metabolic situation of absolute starvation in the neonatal

12 Pettersen J E Formation of *n* hexanedioic acid from hexadecanoic acid by an initial ω-oxidation in ketotic rats *Clin Chim Acta 41* 231 1972
13 Pettersen J E In vitro studies on the metabolism of hexadecanedioic acid and its mono L-carnitine ester *Biochim Biophys Acta 306* 1 1973
14 Shelley H J Carbohydrate reserves in the newborn infant *Br Med J 1* 273 1964
15 Williams L Kaye R & Kumagai M Studies on the mechanism of ketosis in infants and children *Am J Dis Child 94* 499 1957
16 Wada F & Usami M Studies on fatty acid ω-oxidation antiketogenic effect and glyconeogenicity of dicarboxylic acids *Biochim Biophys Acta 487* 261 1977

Submitted Nov 7 1978
Accepted Febr 26 1979

(N G) Department of Clinical Chemistry
Aarhus kommunehospital
DK 8000 Aarhus C
Denmark

Table 1 The urinary excretion of 3 OH butyric acid adipic acid suberic acid and sebacic acid in starved glucose fed and milk fed newborn infants (ranges in mmol/mol creatinine)

Day of life	3 OH butyric acid	Adipic acid	Suberic acid	Sebacic acid
Starved group				
1	ND[a]–14 (2)[b]	7–19 (2)	7–10 (2)	2–4 (2)
2	38–111 (3)	27–65 (3)	8–19 (3)	4–6 (3)
3	50–353 (3)	40–84 (3)	6–22 (3)	4–7 (3)
Glucose fed group				
1	ND–11 (2)	1–4 (2)	2–4 (2)	nd –2 (2)
2	10–26 (2)	nd–5 (2)	1–5 (2)	nd–2 (2)
3	15–17 (2)	2–8 (2)	nd–6 (2)	nd–3 (2)
Mothers milk group				
1	12–15 (2)	8–9 (2)	3–10 (2)	1–4 (2)
2	10–12 (2)	8–9 (2)	4–8 (2)	2–4 (2)
3	29–30 (2)	4–7 (2)	3–4 (2)	2–3 (2)
Normal newborn infants				
1–4	ND–39 (18)	2–48 (10)	2–12 (10)	1–7 (10)
	ND[d]	12[d]	4[d]	3[d]
Normal older children				
2–9 years	14–32 (5)	2–12 (10)	1–6 (10)	1–4 (10)
	17[d]	4[d]	1[d]	2[d]

The figures in parentheses represent the number of children investigated
[a] ND not detectable limit of detection approx 10 mmol/mol creat
nd not detectable limit of detection approx 1 mmol/mol creat
[d] Median values

of succinyl CoA may be a citric acid cycle activity independent of amino acid and carbohydrate catabolism This may explain why some newborns with very low blood glucose do not show clinical signs of hypoglycemia (3 9) and it might be speculated that this metabolic influence of ω oxidation is responsible for the remarkable neonatal resistence to ketosis (6 15)

ACKNOWLEDGEMENT

This study was supported by the Danish Medical Research Council

REFERENCES

1 Bjorkhem I ω-oxidation of stearic acid in the normal starved an diabetic rat liver Eur J Biochem 40 415 1973
2 Bjorkhem I On the mechanism of regulation of ω oxidation of fatty acids J Biol Chem 251 5259 1976
3 Cornblath M Baens G S & Lundeen E Hypoglycemia in premature infants Am J Dis Child 102 729 1961
4 Hemmelgarn E Kumaran K & Landau B R Role of ω-oxidation of fatty acids in formation of the acetyl unit for acetylation J Biol Chem 252 4379 1977
5 Gregersen N Ingerslev J & Rasmussen K Low molecular weight organic acids in the urine of the newborn Acta Paediatr Scand 66 85 1977
6 Heymann W Metabolism studies on age disposition to ketosis in human beings J Pediatr 12 21 1938
7 Mayes P A & Felts J M Regulation of fat metabolism in the liver Nature 215 716 1967
8 McCance R A & Strangeways W M B Protein katabolism and oxygen consumption during starvation in infants young adults and old men Br J Nutr 8 21 1954
9 Pagliara A S Karl I E Haymond M & Kipnis D M Hypoglycemia in infancy and childhood Part I J Pediatr 82 365 1973
10 Pettersen J E Jellum E & Eldjarn L The occurrence of adipic and suberic acids in the urine from ketotic patients Clin Chim Acta 38 17 1972
11 Pettersen J E Urinary excretion of n hexanedioic and n-octanedioic acid in juvenile diabetics with ketonuria Diabetes 23 16 1974

Acta Pædiatr Scand 68 683–690 1979

HYPERLIPOPROTEINEMIA IN NEWBORN INFANTS

A Study of 1025 Families

GUNNAR E ANDERSEN PER LOUS and BENT FRIIS HANSEN

From the Neonatal Department Rigshospitalet and the Department of Clinical Chemistry Bispebjerg Hospital University of Copenhagen Copenhagen Denmark

ABSTRACT Andersen G E Lous P and Friis Hansen B (Neonatal Department Rigshospitalet and the Department of Clinical Chemistry Bispebjerg Hospital Copenhagen Denmark) Hyperlipoproteinemia in newborn infants A study of 1025 families Acta Paediatr Scand 68 683 1979 —As part of a screening study for the detection of hyperlipoproteinemia in 10 000 newborns cord serum lipids and lipoproteins were measured in detail in 1025 infants Elevated cord serum VLDL LDL-cholesterol could easily be identified by a rapid turbidimetric estimation of cord serum VLDL-LDL Cord serum VLDL-LDL-cholesterol was found to be significantly higher than normal in premature asphyxiated and beta methasone phenobarbital ritodrine treated infants Other obstetric complications however were not associated with hyperlipoproteinemia Furthermore all 2050 parents had their serum cholesterol determined 3 parents had familial hypercholesterolemia (FH) One child also had FH though her cord serum total cholesterol and VLDL-LDL-cholesterol were normal The 2 other children of the 3 FH parents had normal lipids and lipoproteins both at birth and follow up

KEY WORDS Cholesterol triglyceride lipoproteins newborns

Within the last few years several studies have been published which suggest that elevated lipids and lipoproteins in newborns may reflect inherited hyperlipoproteinemia (1–5) prematurity (6) postmaturity (7) IUGR (6 8) diabetes in the mother (9–11) perinatal asphyxia (7 12 13) and antepartum betamethasone treatment (14) Since so many conditions in the newborn are associated with elevated cord blood lipids and lipoproteins we have investigated 1) factors during pregnancy and delivery which may affect cord serum lipids 2) the validity of an easy screening method for detecting hyperlipidemia in the newborn 3) if in families with familial hypercholesterolemia examination of cord serum lipids is a reliable way of diagnosing the condition in the infant

MATERIALS

Cord blood was obtained from 1025 newborn infants born in the Rigshospitalet Copenhagen The study and its purpose was explained to the parents and their consent was obtained The obstetric records of all births were studied without prior knowledge of the cord serum lipid and lipoprotein values The data given in Table 1 were collected for each case

Fetal bradycardia is here defined as Either a single episode of FHR<80/min between uterine contractions and/or FHR between 80–1 0/min during more than two uterine contraction cycles and/or abnormal cardiotocographic patterns with late deceleration

The gestational age of low birth weight infants (<2501 g) was assessed by the mother s calculation combined with a clinical examination of the newborn using the technique of Finnstrom (15) In case of uncertainty the gestational age was further compared with the results of an ultrasonic measurement of the biparietal cranial diameter of the fetus If the birth weight was below the 10th percentile value for gestation the infant was classified as SGA

Abbreviations FH = familial hypercholesterolemia AGA = appropriate for gestational age SGA = small for gestational age T C = total cholesterol TG = triglyceride VLDL = very low density lipoproteins LDL = low density lipoproteins HDL = high density lipoproteins VLDL-LDL = VLDL + LDL determined by turbidimetry VLDL LDL-C = VLDL-cholesterol + LDL-cholesterol determined enzymatically after $CaCl_2$ heparin precipitation HDL-C = HDL-cholesterol calculated as the difference (T-C)–(VLDL LDL-C) FHR = fetal heart rate IUGR = intrauterine growth retardation AS = Apgar score

Acta Pædiatr Scand 68 683–690 1979

HYPERLIPOPROTEINEMIA IN NEWBORN INFANTS

A Study of 1 025 Families

GUNNAR E ANDERSEN PER LOUS and BENT FRIIS-HANSEN

From the Neonatal Department Rigshospitalet and the Department of Clinical Chemistry Bispebjerg Hospital University of Copenhagen Copenhagen Denmark

ABSTRACT Andersen G E Lous P and Friis Hansen B (Neonatal Department Rigshospitalet and the Department of Clinical Chemistry Bispebjerg Hospital Copenhagen Denmark) Hyperlipoproteinemia in newborn infants A study of 1025 families Acta Paediatr Scand 68 683 1979 —As part of a screening study for the detection of hyperlipoproteinemia in 10 000 newborns cord serum lipids and lipoproteins were measured in detail in 1 025 infants Elevated cord serum VLDL-LDL-cholesterol could easily be identified by a rapid turbidimetric estimation of cord serum VLDL-LDL Cord serum VLDL-LDL-cholesterol was found to be significantly higher than normal in premature asphyxiated and beta methasone phenobarbital ritodrine treated infants Other obstetric complications however were not associated with hyperlipoproteinemia Furthermore all 2050 parents had their serum cholesterol determined 3 parents had familial hypercholesterolemia (FH) One child also had FH though her cord serum total cholesterol and VLDL-LDL-cholesterol were normal The 2 other children of the 3 FH parents had normal lipids and lipoproteins both at birth and follow up

KEY WORDS Cholesterol triglyceride lipoproteins newborns

Within the last few years several studies have been published which suggest that elevated lipids and lipoproteins in newborns may reflect inherited hyperlipoproteinemia (1–5) prematurity (6) postmaturity (7) IUGR (6 8) diabetes in the mother (9–11) perinatal asphyxia (7 12 13) and antepartum betamethasone treatment (14) Since so many conditions in the newborn are associated with elevated cord blood lipids and lipoproteins we have investigated 1) factors during pregnancy and delivery which may affect cord serum lipids 2) the validity of an easy screening method for detecting hyperlipidemia in the newborn 3) if in families with familial hypercholesterolemia examination of cord serum lipids is a reliable way of diagnosing the condition in the infant

MATERIALS

Cord blood was obtained from 1 025 newborn infants born in the Rigshospitalet Copenhagen The study and its purpose was explained to the parents and their consent was obtained The obstetric records of all births were studied without prior knowledge of the cord serum lipid and lipoprotein values The data given in Table 1 were collected for each case

Fetal bradycardia is here defined as Either a single episode of FHR<80/min between uterine contractions and/or FHR between 80–100/min during more than two uterine contraction cycles and/or abnormal cardiotocographic patterns with late deceleration

The gestational age of low birth weight infants (<2 501 g) was assessed by the mother s calculation combined with a clinical examination of the newborn using the technique of Finnstrom (15) In case of uncertainty the gestational age was further compared with the results of an ultrasonic measurement of the biparietal cranial diameter of the fetus If the birth weight was below the 10th percentile value for gestation the infant was classified as SGA

Abbreviations FH = familial hypercholesterolemia AGA = appropriate for gestational age SGA = small for gestational age T-C = total cholesterol TG = triglyceride VLDL = very low density lipoproteins LDL = low density lipoproteins HDL = high density lipoproteins VLDL-LDL = VLDL + LDL determined by turbidimetry VLDL-LDL-C = VLDL-cholesterol + LDL-cholesterol determined enzymatically after $CaCl_2$ heparin precipitation HDL-C = HDL-cholesterol calculated as the difference (T C)–(VLDL-LDL-C) FHR = fetal heart rate IUGR = intrauterine growth retardation AS = Apgar score

Acta Pædiatr Scand 68 683–690 1979

HYPERLIPOPROTEINEMIA IN NEWBORN INFANTS

A Study of 1025 Families

GUNNAR E ANDERSEN PER LOUS and BENT FRIIS HANSEN

From the Neonatal Department Rigshospitalet and the Department of Clinical Chemistry Bispebjerg Hospital University of Copenhagen Copenhagen Denmark

ABSTRACT Andersen G E Lous P and Friis Hansen B (Neonatal Department Rigshospitalet and the Department of Clinical Chemistry Bispebjerg Hospital Copenhagen Denmark) Hyperlipoproteinemia in newborn infants A study of 1025 families Acta Paediatr Scand 68 683 1979 —As part of a screening study for the detection of hyperlipoproteinemia in 10000 newborns cord serum lipids and lipoproteins were measured in detail in 1025 infants Elevated cord serum VLDL-LDL-cholesterol could easily be identified by a rapid turbidimetric estimation of cord serum VLDL LDL Cord serum VLDL-LDL-cholesterol was found to be significantly higher than normal in premature asphyxiated and betamethasone phenobarbital ritodrine treated infants Other obstetric complications however were not associated with hyperlipoproteinemia Furthermore all 2050 parents had their serum cholesterol determined 3 parents had familial hypercholesterolemia (FH) One child also had FH though her cord serum total cholesterol and VLDL-LDL-cholesterol were normal The 2 other children of the 3 FH parents had normal lipids and lipoproteins both at birth and follow up

KEY WORDS Cholesterol triglyceride lipoproteins newborns

Within the last few years several studies have been published which suggest that elevated lipids and lipoproteins in newborns may reflect inherited hyperlipoproteinemia (1–5) prematurity (6) postmaturity (7) IUGR (6 8) diabetes in the mother (9–11) perinatal asphyxia (7 12 13) and antepartum betamethasone treatment (14) Since so many conditions in the newborn are associated with elevated cord blood lipids and lipoproteins we have investigated 1) factors during pregnancy and delivery which may affect cord serum lipids 2) the validity of an easy screening method for detecting hyperlipidemia in the newborn 3) if in families with familial hypercholesterolemia examination of cord serum lipids is a reliable way of diagnosing the condition in the infant

MATERIALS

Cord blood was obtained from 1025 newborn infants born in the Rigshospitalet Copenhagen The study and its purpose was explained to the parents and their consent was obtained The obstetric records of all births were studied without prior knowledge of the cord serum lipid and lipoprotein values The data given in Table 1 were collected for each case

Fetal bradycardia is here defined as Either a single episode of FHR<80/min between uterine contractions and/or FHR between 80–120/min during more than two uterine contraction cycles and/or abnormal cardiotocographic patterns with late deceleration

The gestational age of low birth weight infants (<2501 g) was assessed by the mother s calculation combined with a clinical examination of the newborn using the technique of Finnstrom (15) In case of uncertainty the gestational age was further compared with the results of an ultrasonic measurement of the biparietal cranial diameter of the fetus If the birth weight was below the 10th percentile value for gestation the infant was classified as SGA

Abbreviations FH = familial hypercholesterolemia AGA = appropriate for gestational age SGA = small for gestational age T-C = total cholesterol TG = triglyceride VLDL = very low density lipoproteins LDL = low density lipoproteins HDL = high density lipoproteins VLDL-LDL = VLDL + LDL determined by turbidimetry VLDL LDL-C = VLDL-cholesterol + LDL-cholesterol determined enzymatically after CaCl heparin precipitation HDL-C = HDL-cholesterol calculated as the difference (T-C) – (VLDL-LDL-C) FHR = fetal heart rate IUGR = intrauterine growth retardation AS = Apgar score

Table 1. *Obstetric data for the 1025 newborns*

Maternal edema (demanding treatment with diuretics)	331	
Maternal proteinuria	15	
Maternal hypertension (130/90 Hg on more than 2 occasions)	37	
Maternal diabetes	23	White group A 12 C 4 B 4 D 3
Betamethasone phenobarbital ritodrine treatment	71	AGA≥37≤42 weeks 23 AGA≥33<37 weeks 24 SGA≥37≤42 weeks 24
Medical induction	138	
Medical stimulation	299	
Sedatives (within 24 hours before delivery)	240	
Analgesia (within 24 hours before delivery)	181	
Prolonged labour (>18 hours)	15	
Prolonged ruptured membranes (>12 hours)	30	
Fever (rectal temperature >38.5 C)	2	
Placenta praevia	5	
Hemorrhage of placenta	6	
Infarction of placenta	51	
Fetal bradycardia (see text)	94	
Abnormal fetal presentation (any other than vertex)	81	(65 breech presentations)
Meconium stained amniotic fluid	112	
Firm cord circumflexion	64	
Caesarean section	143	
Vacuum extraction	135	
Forceps delivery	18	
AS/1 min <7	68	
AS/5 min <7	3	
Boys	531	
Gestational age and maturity	896 47 2 68 12	AGA≥37≤42 weeks AGA≥33<37 weeks AGA<33 weeks SGA≥37≤42 weeks SGA≥33<37 weeks

In order to prevent RDS betamethasone phenobarbital ritodrine treatment was given in 71 cases (Table 1) as described earlier (14). (Ritodrine is a beta adrenergic stimulant which inhibits pre term uterine contractions.) Hereafter this triple drug therapy will be called betamethasone treatment since all evidence points to this drug as having the powerful effect on lipoprotein synthesis.

Subjects. The umbilical cord was clamped and cut within the first 3 min after birth and prior to delivery of the placenta. Mixed arterial and venous blood was allowed to run freely in dry glass tubes, contamination with maternal blood being carefully avoided. The cord blood was stored at 4 C for no longer than 12 hours before serum was separated (2000 r.p.m., 20 min.) and the analyses begun.

Venous blood samples were taken within the first week after delivery from all 2050 parents without prior fasting. Serum TC was determined and if elevated (>95th sex and age corrected percentile value for normal Danes) (4) the serum cholesterol was determined another three to four times after 12 hours fast and in the mothers at least 3 months after the birth had taken place to make sure that the elevation was permanent, and if so the parent and all available family members had their serum lipids and lipoproteins determined.

METHODS

Serum TC was measured manually in duplicate using the enzymatic method described by Roschlau et al. (16). Four Preciset cholesterol standards were included in over 100 separate runs. The coefficient of variation was 4.4% (for the 0.32 mmol/l cholesterol Preciset), 1.7% (for the 2.59 mmol/l cholesterol Preciset), 1.9% (for the 3.88 mmol/l cholesterol Preciset) and 2.1% (for the 10.35 mmol/l cholesterol Preciset).

Serum TG was measured manually in duplicate using the enzymatic method described by Eggstein & Kreutz (17). Two Liponorm triglyceride standards were included in over 100 separate runs. The coefficient of variation was 3.6% (for the 0.42 mmol/l triglyceride Liponorm) and 2.0% (for the 2.50 mmol/l triglyceride Liponorm).

VLDL LDL was measured by a modification of the $CaCl_2$ Heparin turbidimetric method of Burstein & Samaille (18) on a Greiner Selective Analyzer II using 70 µl of serum and 1000 µl of CaCl Heparin precipitation solution. After 5 min at 37°C the sample was read at 578 nm against a sample blank. The concentration of VLDL LDL was expressed in arbitrary units corresponding to the extinction. A frozen (−20°C) stock solution of pooled cord serum (VLDL LDL concentration 45 arbitrary units) was

Table 2 *Percentile values of cord serum lipids and lipoproteins in AGA and SGA infants after uncomplicated delivery (no betamethasone no asphyxia)*

Cord serum	Maturity	No	Cord serum lipid values (mmol/l) 2 5	5	10	50	90	95	97 5
T C	AGA≥37≤42 weeks	117	1 17	1 31	1 38	1 75	2 49	2 83	3 09
VLDL LDL C	AGA≥37≤42 weeks	117	0 39	0 43	0 46	0 72	1 03	1 09	1 40
HDL C	AGA≥37≤42 weeks	117	0 64	0 70	0 76	1 04	1 60	1 74	1 91
TG	AGA≥37≤42 weeks	117	0 18	0 0	0 23	0 39	0 59	0 70	0 75
T-C	AGA≥33<37 weeks	15			1 19	? 33	3 75		
VLDL LDL C	AGA≥33<37 weeks	15			0 56	0 94	1 58		
HDL C	AGA≥33<37 weeks	15			0 63	1 44	? ?1		
TC	AGA≥33<37 weeks	15			0 24	0 30	0 47		
T-C	SGA≥37≤42 weeks	31			1 ?6	1 63	2 14		
VLDL LDL C	SGA≥37≤42 weeks	31			0 55	0 68	1 15		
HDL-C	SGA≥37≤42 weeks	31			0 60	0 93	1 26		
TG*	SGA≥37≤42 weeks	31			0 31	0 55	0 8?		

$p<0.05$ compared with AGA≥37≤42 weeks
$p<0.001$ compared with AGA≥37≤42 weeks

thawed and included in over 100 runs The coefficient of variance was 1 6% VLDL LDL C was measured manually after $CaCl_2$ Heparin precipitation in duplicate as described by Andersen & Gry Nielsen (19) VLDL C was measured manually in duplicate after ultracentrifugation in a 40 3 rotor Beckman type L ultracentrifuge at 10°C at 40 000 r p m for 0 hours Tube slicing was used HDL C values were calculated as the difference between (T C) (VLDL LDL C)

Percentile values were calculated to characterize the distribution of the different lipid and lipoprotein-cholesterol values For comparison of cord serum lipid and lipoprotein-cholesterol values among newborns with a different obstetric history (e g differences in gestational age antepartum drug treatment perinatal asphyxia) the Mann Whitney test was used as described by Siegel (0) The linear relationship between cord serum VLDL LDL C and VLDL LDL was estimated in such a way that the sum of the squared distances from the observations perpendicular to the line was minimized A test of linearity was carried out by dividing the data into 5 equally sized groups at different levels along the line and by performing a t test in each group as well as a combined test of the 5 t values

RESULTS

Factors which may affect cord serum lipids

Of the 1025 deliveries only 163 were uncomplicated In Table 2 the distribution of cord serum lipids and lipoproteins of these infants are given

Cord serum lipids and lipoproteins were compared in 59 normal fullterm boys and 58 normal full term girls no sex difference was found and so the values for all 117 normal boys and girls are here presented together

It is seen that T C and VLDL LDL C levels are higher and TG levels lower in premature newborns (AGA≥33<37 weeks) compared with full term newborns (AGA≥37≤42 weeks) In small for date infants (SGA≥37 ≤42 weeks) HDL C concentrations are lower and TG concentrations higher than in full term infants

The influence of abnormal pregnancy and birth was studied by comparing the distribution of cord serum lipid and lipoprotein values in the 117 normal full term newborns with the distribution in groups of fullterm newborns each group having one of the criteria given in Table 1

In infants who had suffered from perinatal asphyxia serum TG values were higher than in normal newborns The mean TG and p values are given in Table 3 VLDL LDL C followed a similar pattern

Betamethasone treatment was accompanied by hypercholesterolemia in full term and premature infants These results have been presented in detail already (14)

12 newborns of mothers with diabetes mellitus White group A had a median value for cord serum VLDL LDL C of 0 73 mmol/l which is similar to 0 72 mmol/l in normal newborns 11 newborns of mothers with diabetes mellitus White group B–D had a median

Table 1 *Obstetric data for the 1025 newborns*

Maternal edema (demanding treatment with diuretics)	331	
Maternal proteinuria	15	
Maternal hypertension (130/90 Hg on more than 2 occasions)	37	
Maternal diabetes	23	White group A 12 C 4 B 4 D 3
Betamethasone phenobarbital ritodrine treatment	71	AGA≥37≤42 weeks 23 AGA≥33<37 weeks 24 SGA≥37≤42 weeks 24
Medical induction	138	
Medical stimulation	299	
Sedatives (within 24 hours before delivery)	240	
Analgesia (within 24 hours before delivery)	181	
Prolonged labour (>18 hours)	15	
Prolonged ruptured membranes (>12 hours)	30	
Fever (rectal temperature >38 5 C)	2	
Placenta praevia	5	
Hemorrhage of placenta	6	
Infarction of placenta	51	
Fetal bradycardia (see text)	94	
Abnormal fetal presentation (any other than vertex)	81	(65 breech presentations)
Meconium stained amniotic fluid	112	
Firm cord circumflexion	64	
Caesarean section	143	
Vacuum extraction	135	
Forceps delivery	18	
AS/1 min <7	68	
AS/5 min <7	3	
Boys	531	
Gestational age and maturity	896 47 2 68 12	AGA≥37≤42 weeks AGA≥33<37 weeks AGA<33 weeks SGA≥37≤42 weeks SGA≥33<37 weeks

In order to prevent RDS betamethasone phenobarbital ritodrine treatment was given in 71 cases (Table 1) as described earlier (14) (Ritodrine is a beta adrenergic stimulant which inhibits pre term uterine contractions) Hereafter this triple drug therapy will be called betamethasone treatment since all evidence points to this drug as having the powerful effect on lipoprotein synthesis

Subjects The umbilical cord was clamped and cut within the first 3 min after birth and prior to delivery of the placenta Mixed arterial and venous blood was allowed to run freely in dry glass tubes contamination with maternal blood being carefully avoided The cord blood was stored at 4 C for no longer than 12 hours before serum was separated (2 000 r p m 20 min) and the analyses begun

Venous blood samples were taken within the first week after delivery from all 2 050 parents without prior fasting Serum T C was determined and if elevated (>95th sex and age corrected percentile value for normal Danes) (4) the serum cholesterol was determined another three to four times after 12 hours fast and in the mothers at least 3 months after the birth had taken place to make sure that the elevation was permanent and if so the parent and all available family members had their serum lipids and lipoproteins determined

METHODS

Serum T C was measured manually in duplicate using th enzymatic method described by Roschlau et al (16) Fo Preciset cholesterol standards were included in over 10 separate runs The coefficient of variation was 4 4% (f the 0 32 mmol/l cholesterol Preciset) 1 7% (for the 2 mmol/l cholesterol Preciset) 1 9% (for the 3 88 mmol cholesterol Preciset) and 2 1% (for the 10 35 mmol cholesterol Preciset)

Serum TG was measured manually in duplicate usi the enzymatic method described by Eggstein & Kreu (17) Two Liponorm triglyceride standards were include in over 100 separate runs The coefficient of variation wa 3 6% (for the 0 42 mmol/l triglyceride Liponorm) an 2 0% (for the 2 50 mmol/l triglyceride Liponorm)

VLDL LDL was measured by a modification of th $CaCl_2$ Heparin turbidimetric method of Burstein & S maille (18) on a Greiner Selective Analyzer II using 20 µ of serum and 1 000 µl of CaCl Heparin precipitation so lution After 5 min at 37°C the sample was read at 578 n against a sample blank The concentration of VLDL LD was expressed in arbitrary units corresponding to th extinction A frozen (−20°C) stock solution of pooled cord serum (VLDL LDL concentration 45 arbitrary units) was

Table 2 *Percentile values of cord serum lipids and lipoproteins in AGA and SGA infants after uncomplicated delivery (no betamethasone no asphyxia)*

Cord serum	Maturity	No	Cord serum lipid values (mmol/l)						
			2.5	5	10	50	90	95	97.5
T-C	AGA≥37≤42 weeks	117	1.17	1.31	1.38	1.75	2.49	2.83	3.09
VLDL LDL-C	AGA≥37≤42 weeks	117	0.39	0.43	0.46	0.72	1.03	1.09	1.40
HDL C	AGA≥37≤42 weeks	117	0.64	0.70	0.76	1.04	1.60	1.74	1.91
TG	AGA≥37≤42 weeks	117	0.18	0.20	0.23	0.39	0.59	0.70	0.75
T-C	AGA≥33<37 weeks	15			1.19	2.33	3.75		
VLDL LDL C	AGA≥33<37 weeks	15			0.56	0.94	1.58		
HDL-C	AGA≥33<37 weeks	15			0.63	1.44	2.21		
TG	AGA≥33<37 weeks	15			0.24	0.30	0.47		
T-C	SGA≥37≤42 weeks	31			1.26	1.63	2.14		
VLDL LDL C	SGA≥37≤42 weeks	31			0.55	0.68	1.15		
HDL C	SGA≥37≤42 weeks	31			0.60	0.93	1.26		
TG	SGA≥37≤42 weeks	31			0.31	0.55	0.82		

* $p<0.05$ compared with AGA≥37≤42 weeks
** $p<0.001$ compared with AGA≥37≤42 weeks

thawed and included in over 100 runs The coefficient of variance was 1.6% VLDL LDL C was measured manually after $CaCl_2$ Heparin precipitation in duplicate as described by Andersen & Gry Nielsen (19) VLDL C was measured manually in duplicate after ultracentrifugation in a 40.3 rotor Beckman type L ultracentrifuge at 10°C at 40000 r p m for 20 hours Tube slicing was used HDL C values were calculated as the difference between (T C) (VLDL LDL C)

Percentile values were calculated to characterize the distribution of the different lipid and lipoprotein cholesterol values For comparison of cord serum lipid and lipoprotein cholesterol values among newborns with a different obstetric history (e g differences in gestational age antepartum drug treatment perinatal asphyxia) the Mann Whitney test was used as described by Siegel (20) The linear relationship between cord serum VLDL LDL C and VLDL LDL was estimated in such a way that the sum of the squared distances from the observations perpendicular to the line was minimized A test of linearity was carried out by dividing the data into 5 equally sized groups at different levels along the line and by performing a t test in each group as well as a combined test of the 5 t values

RESULTS

Factors which may affect cord serum lipids

Of the 1025 deliveries only 163 were uncomplicated In Table 2 the distribution of cord serum lipids and lipoproteins of these infants are given

Cord serum lipids and lipoproteins were compared in 59 normal fullterm boys and 58 normal full term girls no sex-difference was found and so the values for all 117 normal boys and girls are here presented together

It is seen that T C and VLDL LDL C levels are higher and TG levels lower in premature newborns (AGA≥33<37 weeks) compared with full term newborns (AGA≥37≤42 weeks) In small for date infants (SGA≥37 ≤42 weeks) HDL C concentrations are lower and TG concentrations higher than in full term infants

The influence of abnormal pregnancy and birth was studied by comparing the distribution of cord serum lipid and lipoprotein values in the 117 normal full term newborns with the distribution in groups of fullterm newborns each group having one of the criteria given in Table 1

In infants who had suffered from perinatal asphyxia serum TG values were higher than in normal newborns The mean TG and p values are given in Table 3 VLDL LDL C followed a similar pattern

Betamethasone treatment was accompanied by hypercholesterolemia in full term and premature infants These results have been presented in detail already (14)

12 newborns of mothers with diabetes mellitus White group A had a median value for cord serum VLDL LDL C of 0.73 mmol/l which is similar to 0.72 mmol/l in normal newborns 11 newborns of mothers with diabetes mellitus White group B–D had a median

Table 3 *Comparison of triglyceride values in two subgroups of the infants*
(A) = normal full term newborns without perinatal asphyxia (B) = full term newborns with signs of perinatal asphyxia

Criteria	No	TG median value (mmol/l)	p Value
(A) Normal newborns	117	0 39	
(B) Newborns with perinatal asphyxia			
1 Late decelerations	15	0 67	<0 001
2 FHR <80/min	29	0 57	<0 001
3 FHR <120>80/min	34	0 49	<0 001
4 Meconium stained amniotic fluid	92	0 46	<0 001
5 Firm cord circumflexion	57	0 46	<0 005
6 Vacuum extraction	118	0 46	<0 001
7 AS/1 min <7	41	0 45	<0 05

value of 0 95 mmol/l which is significantly higher than in normal newborns ($p<0\ 005$) This finding however must be interpreted with great caution since the number is small 4 of the 11 were prematurely born and 1 was SGA

The influence of postmaturity could not be investigated since none of the 1 025 newborns were born after the 42nd week of gestation

All of the other criteria presented in Table 1 i e maternal edema proteinuria hypertension infarction and hemorrhage of placenta prolonged ruptured membranes prolonged labour medical induction and stimulation sedatives analgesia abnormal fetal presentation Caesarean section and forceps delivery did not significantly influence the levels of cord serum lipids and lipoproteins

The screening method In Fig 1 the log transformed values of 1025 cord serum VLDL LDL C are plotted against the log transformed values of cord serum VLDL LDL It is seen that all specimens belonging to the upper 5 percent for VLDL LDL C will be included when the upper 15% VLDL LDL values are selected for further study Thus of the 154 newborns in this study with a VLDL LDL concentration above the 85th percentile

Table 4 *Lipid and lipoprotein cholesterol values (mmol/l) in parent child*

Kindred	Age (years)	T C	VLDL LDL C	LDL C	HDL	TG
1 II 8	28	8 97	6 35	6 19	2 62	0 67
1 III 9	At birth	2 05	1 12		1 93	0 77
1 III 9	1 1/12	4 79	3 54	3 30	1 25	0 84
2 III 1	32	9 82	7 75	7 25	2 07	1 74
2 IV 1	At birth	1 70	0 77		0 93	0 63
2 IV 1	1 4/12	2 80	1 59	1 45	1 21	0 85
3 II 1	31	8 72	6 08	5 71	2 64	1 07
3 III 2	At birth	1 49	0 59		0 90	0 31
3 III 2	1 1/12	2 80	1 50	1 15	1 30	0 86
4 II 7	23	9 74	8 62	8 42	1 12	0 89
4 III 2	At birth	2 59	1 38		1 21	0 67
4 III 2	1 5/12	4 70	3 11	2 87	1 59	1 31
5 III 3	33	9 89	7 45	6 80	2 44	1 79
5 IV 2	At birth	1 90	0 75		1 15	0 41
5 IV 2	1 4/12	6 59	4 94	4 38	1 65	1 67

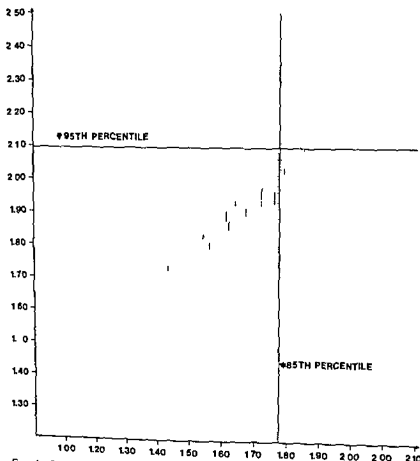


Fig 1 Correlation between 1025 log values for (cord serum VLDL LDL C×100) (*o dinate*) and log values for (cord serum VLDL LDL×100) (abscissa) Three VLDL LDL C values fell on the 95th percentile To visualize these 3 points they have been put above the 95th percentile line Log (VLDL LDL C) = 0 91×log (VLDL LDL)+0 394

47 had VLDL LDL C values above the 95th percentile Follow up values of these 47 infants have been presented in another publication (21)

Diagnosing FH at birth Among the 1025 fathers 55 had hypercholesterolemia (defined as above 95 percentile value for serum T C in normal Danish men (4)) and among the 1025 mothers 137 had hypercholesterolemia within the first week after delivery (defined as above 95 percentile value for serum T C in normal Danish women (4)) These 192 parents were followed and their serum T C was measured another 2–3 times and at least 3 months after delivery In only 3 fathers and 2 mothers a permanent pronounced hypercholesterolemia (over 7 80 mmol/l) was found which gave a suspicion of FH The diagnosis of heterozygous FH is here based upon the finding of a 2–3 fold increase in serum T C and/or LDL C either vertically in three generations or in a family with xanthomatosis The 5 kindreds are depicted in Fig 2 and the corresponding lipid values are given in Table 4 In kindred 1 the child does not have FH His lipids were normal at birth 5 months and 1 1/2 years His father has FH with a type II a pattern In kindred 2 the child does not have FH Her lipids were normal at birth 1 month and 1 4/12 years Her father has FH with a type II a pattern In kindred 3 the child does not have FH His lipids were normal at birth 5 months

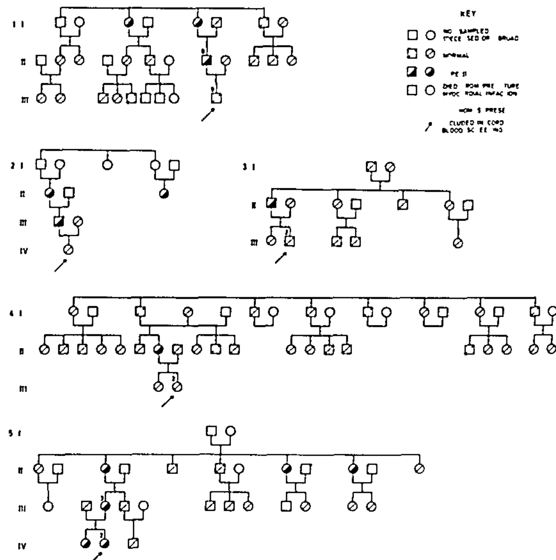


Fig 2 Kindreds

and 1 1/2 years His father has a type II a pattern but he does not have FH since his parents are normal Common causes of secondary hyperlipemia were ruled out by repeated findings of normal glucose tolerance test normal liver thyroid and kidney function tests and normal immunoglobulins In kindred 4 the child does not have FH Her cord serum VLDL LDL C was 1 38 mmol/l which is above the 95th percentile her cord serum T C however was normal = 2 59 mmol/l Her lipoprotein and lipid values were normal at 3 months 1 /2 and 1 5/12 years Her mother has a type II a pattern, but she does not have FH Also in this case common causes of secondary hyperlipemia were ruled out In kindred 5 the child has FH with elevated lipids at age 3 months 1 1/2 and 1 4/2 years her cord serum T C and VLDL LDL C however were normal Her mother has FH with a type II a pattern The mother developed abnormal glucose tolerance during pregnancy which again became normal after delivery

DISCUSSION

In our 117 normal newborns the median concentrations of cord serum T C VLDL LDL C HDL C and TG were almost similar to the mean concentrations found by Glueck et al (22) Kwiterovich et al (1) Ose et al (23) and Andersen & Friis Hansen (6) With one exception (22) the 95 percentile cut off value for cord serum VLDL LDL C seems to be be

tween 1 09–1 27 mmol/l for normal newborns The present finding of higher than normal cord serum T C VLDL LDL C and HDL C values in premature newborns (⩾33<37 weeks of gestation) confirms the results of Sabata (24) Ose et al (23) and Andersen & Friis Hansen (6) but is different from the results obtained by Fosbrooke & Wharton (8) who in 16 pre mature newborns (28–36 weeks of gestation) found the same levels of cord serum T C as in full term newborns The reason for this might be their inclusion of premature new borns of under 33 weeks of gestation We suggest that during the maximal growth rate period between the 33nd and 37th week of gestation during which the fetus will increase its weight by about 200 g/week the need of particularly hepatic VLDL LDL C for mem brane and hormone synthesis by non hepatic parenchymal cells is increased During the decelerating growth rate period after the 37th week of gestation during which the weekly increase in fetal weight averages only 70 g the serum concentration of T C VLDL LDL C and HDL C falls corresponding to lower cord serum values in full term compared to prema ture newborns Our finding of elevated cord serum TG in SGA infants born after IUGR is in agreement with the results of Fosbrooke & Wharton (8) and Andersen & Friis Hansen (6) Also do our findings of elevated TG and VLDL LDL C in newborns with perinatal as phyxia confirm the results reported by Tsang et al (12) Andersen & Friis Hansen (13) and Cress et al (7)

In an earlier publication (19) we have shown that there is a good correlation between the concentration of cholesterol in VLDL+LDL isolated in the ultracentrifuge and in VLDL LDL after heparin $CaCl_2$ precipitation In the present study a good linear correlation was found between VLDL LDL C and VLDL LDL thus indicating that for screening pur poses the rapid and inexpensive turbidimetric determination of VLDL LDL can be used in stead of the more laborious and expensive measurement of VLDL LDL C The draw back of the screening method is the necessity to include as much as 15% of the specimens in a more elaborate analytical procedure to be sure to select the 5% with the highest VLDL LDL C values

The possible diagnosis of FH in newborns has been a matter of debate within the last few years The interest in making an early diagnosis has to do with the theoretical benefit in reducing the increased risk of premature coronary heart disease by early dietary and pharmaceutical intervention Five studies (1 2 4 25 26) have shown that measurement of cord serum T C alone does not discriminate newborns with FH This is probably due to the fact that most of the cord serum T C is not transported by the LDL but by the HDL fraction Four studies (1–4) have shown that an elevation of cord serum LDL or VLDL+ LDL will give a suspicion of FH in the child but only one study (1) has unambiguously shown that an elevated cord serum LDL C combined with a follow up study after year one in a child with known FH in one parent allows a diagnosis of FH In children with un known parental phenotypes the three existing studies have been incomplete and inconclusive as for estimation of incidence of FH and false positive and negative diagnosis Greten et al (2) in 1 323 children found 6 (0 45%) having an elevated VLDL LDL C at birth and after age one but the diagnosis FH was not verified since no family studies were carried out Tsang et al (3 22) in 1 800 consecutively born infants found 3 (0 17%) having an elevated (>95th percentile value) LDL C at birth and after age one combined with a three genera tion vertical transmission of elevated T C in one parent and grandparent However only newborns with an elevated T C had their cord serum LDL C determined Andersen & Friis-Hansen (4) among 303 full term newborns found 3 (0 99%) with an elevated (>97 5th per centile) cord serum prebeta betalipoprotein value who at follow up after year one and by three generation vertical family studies were shown to have FH This study however did

not include measurement of cord serum VLDL LDL C and the newborns were all full term

In the present study of 1 025 infants 1 child (0 10%) had FH but this diagnosis was not suspected at birth since her cord serum VLDL LDL C was normal The reason why she did not have elevated VLDL LDL C at birth is unclear The abnormal glucose tolerance found in the mother just prior to birth could hardly explain this finding of normal cord serum lipids Glueck (27) also has studied a child with FH who had normal cord serum lipid and lipoprotein values of which no explanation could be given

ACKNOWLEDGEMENTS

This study was supported by grants from the Danish Heart Foundation and the Dagmar Marshall Fund

REFERENCES

1 Kwiterovich P O Levy R I & Fredrickson D S Neonatal diagnosis of familial type II hyperlipoproteinemia *Lancet I* 118 1973
2 Greten H Wagner M & Schettler G Fruhdiagnose und Haufigkeit der familiaren Hyperlipoproteinamie Typ II *Dtsch Med Wochenschr 99* 2553 1974
3 Tsang R C Glueck C J Fallat R W & Mellies M Neonatal familial hypercholesterolemia *Am J Dis Child 129* 83 1975
4 Andersen G E & Friis Hansen B Neonatal diagnosis of familial type II hyperlipoproteinemia *Pediatrics 57 214 1976*
5 Glueck C J Fallat R W Millett F & Steiner P M Familial hyperalphalipoproteinemia *Acta Int Med 135* 1025 1975
6 Andersen G E & Friis Hansen B Cord serum lipid and lipoprotein cholesterol values in normal and betamethasone treated newborns of varying gestational age *Acta Paediatr Scand 66* 355 1977
7 Cress H R Shaher R M Laffin R & Karpowicz K Cord blood hyperlipoproteinemia and perinatal stress *Pediatr Res 11 19 1977*
8 Fosbrooke A S & Wharton B A Plasma lipids in umbilical cord blood from infants of normal and low birth weight *Biol Neonate 23* 330 1973
9 Mortimer J G Cord blood lipids of normal infants and infants of diabetic mothers *Arch Dis Child 39* 342 1964
10 Pantelakis S N Cameron A H Davidson S Dunn P M Fosbrooke A S Lloyd J K Malins J M & Wolff O H The diabetic pregnancy A study of serum lipids in maternal and umbilical cord blood and of the uterine and placental vasculature *Arch Dis Child 39* 334 1964
11 Lloyd A V Observations on the composition of the blood in the neonatal period Thesis University of Edinburgh 1963
12 Tsang R C Glueck C J Evans G & Steiner P M Cord blood hypertriglyceridemia *Am J Dis Child 127* 78 1974
13 Andersen G E & Friis Hansen B Neonatal hypertriglyceridemia *Acta Paediatr Scand 65* 369 1976
14 Andersen G E & Friis Hansen B Hypercholesterolemia in the newborn after antepartum treatment with betamethasone phenobarbital ritodrine for the prevention of the respiratory distress syndrome *Pediatrics 62* 8 1978
15 Finnström O Studies on maturity in newborn infants II External characteristics *Acta Paediatr Scand 61* 24 1972
16 Roschlau P Bernt E & Gruber W Enzymatische Bestimmung des Gesamt Cholesterins im Serum *Z Klin Chem Klin Biochem 12* 403 1974
17 Eggstein M & Kreutz F H Eine neue Bestimmung der Neutralfette im Blutserum und Gewebe *Klin Wochenschr 44* 262 1966
18 Burstein M & Samaille J Nouvelle methode de séparation et de dosage des lipoproteines de faible densité *Ann Biol Clin 17* 23 1959
19 Andersen G E & Gry Nielsen H Neonatal screening for hyperlipoproteinemia Methods for direct estimation of cord serum VLDL+LDL *Clin Chim Acta* 66 29 1976
20 Siegel S *In* S Siegel (ed) *Nonparametric statistics for the behavioral sciences* McGraw Hill New York 1956 p 116
21 Andersen G E Lous P & Friis Hansen B Screening for hyperlipoproteinemia in 10 000 Danish newborns Follow up studies in 52 children with elevated cord serum VLDL LDL cholesterol *Acta Paediatr Scand 68* 541 1979
22 Glueck C J Heckman F Schoenfeld M Steiner P & Pearce W Neonatal familial type II hyperlipoproteinemia Cord blood cholesterol in 1800 births *Metabolism 20* 597 1971
23 Ose L Bakke T Iden A & Aarskog D Neonatal screening for hyperlipidaemia *Postgrad Med J 51* (Suppl 8) 88 1975
24 Šabata V Carbohydrate and lipid metabolism of the human fetus *Rozpravy Československé Akademie Ved 83* 9 1973
25 Darmady J M Fosbrooke A S & Lloyd J K Prospective study of serum cholesterol levels during first year of life *Br Med J II* 685 1972
26 Potter J M & Nestel P J Comparison of plasma lipids at birth and in second year of life *Aust N Z J Med 6* 420 1976
27 Glueck C J Personal communication

Submitted Jan 27 1979
Accepted March 14 1979

(G E A) Neonatal Department GN 5024
Rigshospitalet
Blegdamsvej 9
DK 2100 Copenhagen
Denmark

Acta Pædiatr Scand 68 691–694 1979

PROSPECTIVE STUDIES OF THE EFFECT OF BREAST FEEDING ON INCIDENCE OF INFECTION AND ALLERGY

R K CHANDRA

From the Department of Pediatrics Memorial University of Newfoundland St John s Newfoundland Canada

ABSTRACT Chandra R K (Department of Pediatrics Memorial University of Newfoundland St John s Newfoundland Canada) Prospective studies of the effect of breast feeding on incidence of infection and allergy Acta Paediatr Scand 68 691 1979 —The effect of exclusive breast feeding in the first few weeks after birth on infant morbidity due to infectious and allergic disorders was investigated in three separate prospective studies In a rural community in India breast fed infants had a significantly lower incidence of respiratory infection otitis diarrhoea dehydration and pneumonia In an urban population in Canada breast feeding was associated with a marked decrease in the occurrence of otitis and respiratory disease and to a lesser extent of diarrhoea and dehydration In newborn siblings of children with atopic disease exclusively breast fed for a minimum of six weeks the incidence of eczema recurrent wheezing elevated serum IgE IgE-antibodies to cow s milk complement activation in vivo after milk challenge and hemagglutinating antibodies to β-lactoglobulin was significantly lower compared with formula fed matched group These observations provide clinical data attesting the immunologic advantages of human milk

KEY WORDS Breast feeding human milk infection allergic disease morbidity eczema asthma immunoglobulin E

The deleterious effects of artificial feeding techniques supplanting breast feeding have been documented repeatedly Microbial colonization and infection related morbidity is reported to be lower in infants fed human milk compared with those fed on cow s milk formula (6 10–12 21 25 28 29) However many of the studies showing health advantage for breast fed infants relied on anecdotal clinical experience medical records and retrospective data (see 6) Recently elaborate demonstration of a variety of protective factors in human milk has provided sound immunologic evidence of the beneficial effects of breast feeding In this paper controlled prospective observations indicate that breast feeding is associated with a reduction in the incidence of infections and of allergy

MATERIAL AND METHODS

I I ti n-r lated morbid n

Ind a Thirty five newborn infants fed exclusively on the breast for at least the first ? months of l fe were studied The average duration of exclusive breast feeding was 4 8 months (range 2 ?–8 5 months) The age upto which breast feeding was continued ranged from 4 to 24 months (mean 9 5 months) A trained auxillary nurse midwife visited ea h family once a week and recorded morbidity data Thirty five controls bottle fed on fresh cow s or buffalo s milk from the first week onwards were similarly observed The two groups were matched for socioeconomic status parental education and occupation and family size Respiratory infection was defined as cough during the day and night for 7 hours or more with or without fever or corhyza Otitis was defined as mucopurulent discharge from external auditory meatus with or without fever irritability and pulling at ears Diarrhoea was defined as 3 or more loose bowel movements for 48 hours or longer Dehydration was diagnosed on physi al examination and pneumonia by radiology

Canada Thirty neonates fed exclusively on the breast for at least the first two months of life were followed up for a period of 24 months The average duration of exclusive breast feeding was 3–6 months (range 2 5–5 8 months) The age up to which breast feeding was continued ranged from 4 to 14 months (mean 7 5 months) Thirty infants fed on cow s milk formula (Carnation) from the first day onwards acted as controls The two groups were matched for socioeconomic status parental education and family size The parents were contacted by telephone once in two weeks and morbidity data recorded In most instances the diagnosis of illness was confirmed on physical examination

not include measurement of cord serum VLDL LDL C and the newborns were all full term

In the present study of 1 025 infants 1 child (0 10%) had FH but this diagnosis was not suspected at birth since her cord serum VLDL LDL C was normal The reason why she did not have elevated VLDL LDL C at birth is unclear The abnormal glucose tolerance found in the mother just prior to birth could hardly explain this finding of normal cord serum lipids Glueck (27) also has studied a child with FH who had normal cord serum lipid and lipoprotein values of which no explanation could be given

ACKNOWLEDGEMENTS

This study was supported by grants from the Danish Heart Foundation and the Dagmar Marshall Fund

REFERENCES

1 Kwiterovich P O Levy R I & Fredrickson D S Neonatal diagnosis of familial type II hyperlipoproteinemia *Lancet I* 118 1973
2 Greten H Wagner M & Schettler G Fruhdiagnose und Haufigkeit der familiaren Hyperlipoproteinamie Typ II *Dtsch Med Wochenschr* 99 2553 1974
3 Tsang R C Glueck C J Fallat R W & Mellies M Neonatal familial hypercholesterolemia *Am J Dis Child* 129 83 1975
4 Andersen G E & Friis Hansen B Neonatal diagnosis of familial type II hyperlipoproteinemia *Pediatrics* 57 214 1976
5 Glueck C J Fallat R W Millett F & Steiner P M Familial hyperalphalipoproteinemia *Acta Int Med* 135 1025 1975
6 Andersen G E & Friis Hansen B Cord serum lipid and lipoprotein cholesterol values in normal and betamethasone treated newborns of varying gestational age *Acta Paediatr Scand* 66 355 1977
7 Cress H R Shaher R M Laffin R & Karpowicz K Cord blood hyperlipoproteinemia and perinatal stress *Pediatr Res* 11 19 1977
8 Fosbrooke A S & Wharton B A Plasma lipids in umbilical cord blood from infants of normal and low birth weight *Biol Neonate* 23 330 1973
9 Mortimer J G Cord blood lipids of normal infants and infants of diabetic mothers *Arch Dis Child* 39 342 1964
10 Pantelakis S N Cameron A H Davidson S Dunn P M Fosbrooke A S Lloyd J K Malins J M & Wolff O H The diabetic pregnancy A study of serum lipids in maternal and umbilical cord blood and of the uterine and placental vasculature *Arch Dis Child* 39 334 1964
11 Lloyd A V Observations on the composition of the blood in the neonatal period Thesis University of Edinburgh 1963
12 Tsang R C Glueck C J Evans G & Steiner P M Cord blood hypertriglyceridemia *Am J Dis Child* 127 78 1974
13 Andersen G E & Friis Hansen B Neonatal hypertriglyceridemia *Acta Paediatr Scand* 65 369 1976
14 Andersen G E & Friis Hansen B Hypercholesterolemia in the newborn after antepartum treatment with betamethasone phenobarbital ritodrine for the prevention of the respiratory distress syndrome *Pediatrics* 62 8 1978
15 Finnstrom O Studies on maturity in newborn infants II External characteristics *Acta Paediatr Scand* 61 24 1972
16 Roschlau P Bernt E & Gruber W Enzymatische Bestimmung des Gesamt Cholesterins im Serum *Z Klin Chem Klin Biochem* 12 403 1974
17 Eggstein M & Kreutz F H Eine neue Bestimmung der Neutralfette im Blutserum und Gewebe *Klin Wochenschr* 44 262 1966
18 Burstein M & Samaille J Nouvelle methode de séparation et de dosage des lipoprotéines de faible densité *Ann Biol Clin* 17 23 1959
19 Andersen G E & Gry Nielsen H Neonatal screening for hyperlipoproteinemia Methods for direct estimation of cord serum VLDL+LDL *Clin Chim Acta* 66 29 1976
20 Siegel S *In* S Siegel (ed) *Nonparametric statistics for the behavioral sciences* McGraw Hill New York 1956 p 116
21 Andersen G E Lous P & Friis Hansen B Screening for hyperlipoproteinemia in 10 000 Danish newborns Follow up studies in 522 children with elevated cord serum VLDL LDL cholesterol *Acta Paediatr Scand* 68 541 1979
22 Glueck C J Heckman F Schoenfeld M Steiner P & Pearce W Neonatal familial type II hyperlipoproteinemia Cord blood cholesterol in 1800 births *Metabolism* 20 597 1971
23 Ose L Bakke T Iden A & Aarskog D Neonatal screening for hyperlipidaemia *Postgrad Med J* 51 (Suppl 8) 88 1975
24 Šabata V Carbohydrate and lipid metabolism of the human fetus *Rozpravy Československe Akademie Ved* 83 9 1973
25 Darmady J M Fosbrooke A S & Lloyd J K Prospective study of serum cholesterol levels during first year of life *Br Med J II* 685 1972
26 Potter J M & Nestel P J Comparison of plasma lipids at birth and in second year of life *Aust N Z J Med* 6 420 1976
27 Glueck C J Personal communication

Submitted Jan 27 1979
Accepted March 14 1979

(G E A) Neonatal Department GN 5024
Rigshospitalet
Blegdamsvej 9
DK 2100 Copenhagen
Denmark

Table 3 *Incidence of eczema, recurrent wheezing and in vitro correlates of allergy in breast fed and formula fed infants*

Figures refer to the number of infants affected or showing positive test

Parameter	Breast fed ($n=37$)	Formula fed ($n=37$)	P
Eczema	4	21	<0.001
Recurrent wheezing	1	8	<0.01
Serum IgE >60 IU/ml	6	29	<0.001
IgE antibodies to cow's milk	1	15	<0.001
Complement activation in vivo after milk challenge	0	6	<0.001
Hemagglutinating antibodies to β lactoglobulin	3	31	<0.05
Eosinophilia 400 per mm^3	0	5	<0.05

been detected and are functionally effective in the gut lumen against the respective micro-organisms and their products (15, 18, 20, 24). There is recent evidence to suggest that colostral antibodies can be absorbed by the healthy newborn for the first three days after birth and by the low birth weight infant for several weeks (R. K. Chandra, unpublished data). The antibody content of milk reflects the immunologic experience of the mother's gastrointestinal tract (19), and may be mediated by unique characteristics of lymphocyte traffic in the enteric circulation and lymphatics. T and B lymphocytes, neutrophils and macrophages are present and immunocompetent (3, 6, 24; R. K. Chandra, unpublished data). Furthermore, antigen nonspecific resistance factors such as lactoferrin, lysozyme and interferon provide additional antimicrobial activity in breast milk (1, 14). These many unique properties of human milk provide distinct advantages to the breast fed infant in terms of morbidity experience and survival, particularly when children are raised in less than ideal circumstances (4, 8). Additionally, nutritional deprivation due to poverty and contamination of the formula by enteropathogens as the result of poor hygiene and inadequate cleaning and storage facilities may increase the chances of gastrointestinal infections in the bottle fed infant.

The protective effect of breast feeding in the prevention of allergy was clearly established in our study. Differences in incidence of clinical atopic disease and complementary laboratory evidence in the breast fed and artificially fed groups (Table 3) strongly incriminate cow's milk formula in the genesis of hypersensitivity in susceptible infants. This lends further support to our previous data (5, 7) and recent reports of other studies (13). Mathew et al. (22) followed up infants of atopic parents and observed a dramatic reduction in the incidence of clinical eczema in the group exclusively breast fed for the first 12 weeks of life. Positive skin reactions to environmental inhalants and ingestants were seen less frequently than in the formula fed control group. The increased permeability of the neonatal intestine to dietary macromolecules provides an avenue for initial sensitization to food allergens. The duration of the postnatal period of such susceptibility is not established and may well be only a few weeks, differing in healthy and growth retarded infants and reappearing in the older infant during the acute and convalescent phases of viral gastroenteritis (R. K. Chandra, unpublished data). The subject of breast feeding and common atopic disease has been reviewed elsewhere (6, 27). The literature suggests that exclusive breast feeding will reduce the incidence of allergy in the at risk infant. Our data shows that six weeks of exclusive breast feeding is effective in impairing the development of hypersensitivity and in lowering the incidence of manifest allergic disease in vulnerable infants.

Table 1 *Infection related morbidity in breast fed and formula fed infants in India*

The data is expressed as number of episodes of illness for the group over a 12 month period n=number of infants in each group *Differences between the two groups were* analyzed by χ^2 test

Disorder	Breast fed (n=35)	Formula fed (n=35)	p
Respiratory infection	57	109	<0 001
Otitis	21	52	<0 001
Diarrhoea	70	211	<0 001
Dehydration	3	14	<0 001
Pneumonia	2	8	<0 001

Allergy

Thirty seven infants with an older sibling diagnosed to have atopic disease were exclusively fed on the breast for the first six weeks of life or longer During this period no supplements other than water were given Each infant was examined at 3 month intervals during the first year and thereafter at 6-month intervals till the age of 3 years All children were followed up for a minimum of 24 months (median 27 8 months) Another group of 37 infants who also had an older sibling with allergic disease but were fed cow s milk formula were similarly examined The two groups did not differ significantly in any other respect Identical advice concerning the nature and the age of introduction of food supplements was offered to all the families Blood was collected at the ages of 12 and 24 months IgE was estimated by radioimmunoassay (Pharmacia) IgE antibodies by the radioallergosorbent test (Pharmacia) and antibodies to β lactoglobulin by tanned red cell hemagglutination (2) In addition evidence for complement activation after cow s milk challenge was sought by immunoelectrophoresis and complement C3 concentration (23) Eczema was diagnosed on the basis of characteristic distribution and morphology of skin lesions Wheezing was considered to be of allergic etiology if 3 or more episodes had occurred absence of fever at onset and dramatic response to sympathomimetic agents like adrenaline and orciprenaline

RESULTS

There was a significant difference in infectious morbidity among breast fed and bovine milk fed infants both in India (Table 1) and in Canada (Table 2) There was a lower incidence of respiratory and diarrhoeal disease and of complications such as pneumonia and dehydration Among urban Canadian infants there was a dramatic beneficial effect of breast feeding on occurrence of middle ear infection a reduction of almost 10 fold was observed

In infants with family history of atopy exclusive breast feeding for a period of 4 weeks or longer was associated with a marked reduction in the incidence of clinical atopic eczema and of recurrent allergic wheezing (Table 3) A larger proportion of formula fed infants had high levels of serum IgE Eosinophilia was seen in 6 of 37 artificially fed infants and in none of the breast fed babies IgE antibodies to cow s milk proteins were detected in 40% of the formula fed group and in 3% of the breast fed group and hemagglutinating antibodies to β lactoglobulin were observed in 84% and 8% respectively Complement activation *in vivo* after milk challenge was found only in some of the formula fed infants

DISCUSSION

Our controlled prospective studies one in a developing country and the other in an industrialized country have confirmed the significant beneficial effects of breast feeding on infection related morbidity Many of the previous reports critically analyzed elsewhere (6) were based on retrospective data and had failed to provide incontrovertible evidence on the subject Many different proteins enzymes and cells constitute host resistance factors in the human colostrum and milk (6 9 14 17 19 26) Secretory antibodies against a variety of viruses enterobacteria and enterotoxins have

Table 2 *Infection related morbidity in breast fed and formula fed infants in Canada*

The data is expressed as number of episodes of illness for the group over a 24 month period n=number of children in each group Differences between the two groups were *analyzed by χ^2 test*

Disorder	Breast fed (n=30)	Formula fed (n=30)	p
Respiratory infection	42	98	<0 001
Otitis	9	86	<0 001
Diarrhoea	5	16	<0 01
Dehydration	0	3	0 05<p<0 1

Acta Pædiatr Scand 68 695–700 1979

SERUM IgA IN THE NEONATE

Molecular Size Concentration and Effect of Breast Feeding

P L YAP A PRYDE P J LATHAM and D B L McLELLAND

From the MRC Unit of Reproductive Biology the Department of Therapeutics University of Edinburgh Royal Infirmary the Neonatal Unit Simpson Maternity Memorial Pavilion and the Blood Transfusion Service Royal Infirmary Edinburgh Scotland

ABSTRACT Yap P L Pryde A Latham P J and McClelland D B L (MRC Unit of Reproductive Biology 2 Forrest Road Edinburgh Department of Therapeutics Edinburgh University Neonatal Unit Simpson Memorial Maternity Pavilion and Blood Transfusion Service Royal Infirmary Edinburgh) Serum IgA in the neonate Molecular size concentration and effect of breast feeding Acta Paediatr Scand 68 695 1979.—IgA concentrations in the serum of 48 six day-old neonates (23 exclusively artificially fed 25 exclusively breast fed) were measured using a double antibody radioimmunoassay In 24 of the neonates umbilical cord blood was also studied Gel filtration was used to estimate the molecular size of IgA present in cord and neonatal serum The arithmetic mean concentration of IgA (±S E M) found in the 48 neonates was 2 6±1 45 mg/l No significant difference was detected between the breast fed and artificially fed neonates Only 7S IgA was detected in cord blood and in neonatal serum Six days of exclusive breast feeding therefore has no influence on total serum IgA levels on the sixth day of neonatal life nor does it result in detectable circulating 11S IgA at that time

KEY WORDS Serum IgA 6-day-old neonate breast feeding

The human Immunoglobulin A system is immature at birth (15) However the breast fed neonate receives by mouth large quantities of secretory IgA in the form of colostrum and milk (8 12) The IgA in colostrum and milk differs from the IgA found in adult serum consisting largely of a dimer with a sedimentation coefficient of 11S and molecular weight of 370000 compared with serum IgA which is a 7S monomer of molecular weight 170000 (5 9) This is mainly due to the presence of an additional protein secretory component which in combination with two molecules of monomeric IgA results in a dimer that is more resistant to digestion by intestinal proteolytic enzymes (2) Although it is known that the majority of IgA in feeds of colostrum and milk is not absorbed by the infant (1 7 10) it has been suggested that the gut in early neonatal life is capable of absorbing small quantities of IgA (6 10) However the assay techniques which have been used have been relatively insensitive and it was considered desirable to examine this question using a very sensitive assay in a study of normally breast fed infants

The purpose of this study was to observe if exclusive breast feeding for 1 week produced a detectable effect on serum IgA levels in the neonate We have compared by means of a specific IgA radioimmunoassay the serum IgA concentrations in neonates after six days of exclusive breast feeding with serum IgA concentrations in neonates that were exclusively artificially fed In addition we have studied the metabolism of IgA in early life by analysing cord blood samples and comparing IgA concentrations present at birth with those found in the same infant six days later We have also estimated by gel filtration the molecular size of IgA in the neonate

ACKNOWLEDGEMENTS

I thank the many physicians for cooperation and referrals Gregg Woodford and Joan Dwyer provided excellent technical help Antoinette Smith helped in preparation of the manuscript

REFERENCES

1 Bullen J J Rogers H J & Leigh L Iron binding protein in milk and resistance to *Escherichia coli* infection in infants *Br Med J I* 69 1972
2 Chandra R K Food antibodies in malnutrition *Arch Dis Child 50* 532 1975
3 Chandra R K Nutrition dietary factors and immunology of the gut In *Tripartite Symposium on Gastroenterology* City University London 1977
4 Chandra R K Basis for possible interventions to improve the immune response in the malnourished In *International Symposium on Nutritional Interventions* Instituto de Nutrición y Technología de los Alimentos Universidad de Chile Santiago 1978
5 Chandra R K Breast feeding and prevention of atopic eczema *Pediatr Res 12* 478 1978
6 Chandra R K Immunological aspects of human milk *Nutr Rev 36* 265 1978
7 Chandra R K Nutritional deficiency and susceptibility to infection *Bull WHO 57* 167 1979
8 Chandra R K & Newberne P M *Nutrition immunity and infection Mechanisms of interactions* Plenum New York 1977
9 Chandra R K Mandeville R P & Dearlove J What s new in clinical immunology *In* L S Prasad (ed) *Paediatric Problems in South East Asia* Sanjivan Press Patna 1977 p D1
10 Crosse V M *The premature baby* Churchill London 1966 p 143
11 Cunningham A S Morbidity in breast fed and artificially fed infants *J Pediatr 90* 726 1977
12 Downham M A P S Scott Sims D G Webb J K G & Gardner P S Breast feeding protects against respiratory syncytial virus infections *Br Med J II* 274 1976
13 Editorial Towards prevention of allergy *Lancet I* 339 1977
14 Goldman A S & Smith C W Host resistance factors in human milk *J Pediatr 82* 1082 1973
15 Gothefors L Studies of antimicrobial factors in human milk and bacterial colonization of the new born Medical dissertation Umeå Universitet Umeå 1975
16 Gothefors L Olling S & Winberg J The effect of human milk on the intestinal flora Relevance for host resistance In *Swedish Nutrition Foundation Symposium on Food and Immunology* Almqvist & Wiksell Stockholm 1977 p 154
17 Hanson L Å & Winberg J Breast milk and defense against infection in the newborn *Arch Dis Child 47* 845 1972
18 Hanson L Å Ahlstedt S Carlsson B Goldblum R M Linblad B S & Kaijser B The antibodies of hum in milk their origin and specificity In *Swedish Nutrition Foundation Symposium on Food and Immunology* Almqvist & Wiksell Stockholm 1977 p 148
19 Hanson L Å Ahlstedt S Carlsson B Fällström S P Kaijser B Lindblad B S Sohl Åkerlund A & Svanborg Eden C New Knowledge in human milk immunology *Acta Paediatr Scand 67* 577 1978
20 Holmgren J Hanson L Å Carlsson B Lindblad B S & Rahimtoola J Neutralizing antibodies against *Escherichia coli* and *Vibrio cholerae* enterotoxins in human milk in a developing country *Scand J Immunol 5* 867 1976
21 Mata L J & Urrutia J J Intestinal colonization of breast fed children in a rural area of low socioeconomic level *Ann N Y Acad Sci 176* 93 1971
22 Mathew D J Taylor B Norman A P Turner M W & Soothill J F Prevention of eczema *Lancet I* 321 1977
23 Matthews T S & Soothill J F Complement activation after milk feeding in children with cow s milk allergy *Lancet II* 893 1970
24 Ogra S S & Ogra P L Immunologic aspects of human colostrum and milk *J Pediatr 92* 546 550 1978
25 Plank S J & Milanesi M L Infant feeding and infant mortality in rural Chile *Bull WHO 48* 03 1973
26 Reddy V Bhaskaram C Raghuramulu N & Jagadeesan V Antimicrobial factors in human milk *Acta Paediatr Scand 66* 229 1977
27 Soothill J F Immunodeficiency allergy and infant feeding In *Swedish Nutrition Foundation Symposium on Food and Immunology* Almqvist & Wiksell Stockholm 1977 p 88
28 Tassovatz B & Kotsitch A Le lait de femme et son action de protection contre les infections intestinales chez le nouveau ne *Ann Pediat 37* 1649 1961
29 Winberg J & Wessner G Does breast milk protect against septicaemia in the newborn? *Lancet I* 1091 1971

Submitted March 12 1979
Accepted March 28 1979

Department of Nutrition & Food Science
Massachusetts Institute of Technology
Cambridge Mass 02139
USA

SERUM IgA IN THE NEONATE

Molecular Size Concentration and Effect of Breast Feeding

P L YAP A PRYDE P J LATHAM and D B L McLELLAND

From the MRC Unit of Reproductive Biology the Department of Therapeutics University of Edinburgh Royal Infirmary the Neonatal Unit Simpson Maternity Memorial Pavilion and the Blood Transfusion Service Royal Infirmary Edinburgh Scotland

ABSTRACT Yap P L Pryde A Latham P J and McClelland D B L (MRC Unit of Reproductive Biology 2 Forrest Road Edinburgh Department of Therapeutics Edinburgh University Neonatal Unit Simpson Memorial Maternity Pavilion and Blood Transfusion Service Royal Infirmary Edinburgh) Serum IgA in the neonate Molecular size concentration and effect of breast feeding Acta Paediatr Scand 68 695 1979 —IgA concentrations in the serum of 48 six-day-old neonates (23 exclusively artificially fed 25 exclusively breast fed) were measured using a double antibody radioimmunoassay In 24 of the neonates umbilical cord blood was also studied Gel filtration was used to estimate the molecular size of IgA present in cord and neonatal serum The arithmetic mean concentration of IgA (±S E M) found in the 48 neonates was 2 6±1 45 mg/l No significant difference was detected between the breast fed and artificially fed neonates Only 7S IgA was detected in cord blood and in neonatal serum Six days of exclusive breast feeding therefore has no influence on total serum IgA levels on the sixth day of neonatal life nor does it result in detectable circulating 11S IgA at that time

KEY WORDS Serum IgA 6-day-old neonate breast feeding

The human Immunoglobulin A system is immature at birth (15) However the breast fed neonate receives by mouth large quantities of secretory IgA in the form of colostrum and milk (8 12) The IgA in colostrum and milk differs from the IgA found in adult serum consisting largely of a dimer with a sedimentation coefficient of 11S and molecular weight of 370000 compared with serum IgA which is a 7S monomer of molecular weight 170000 (5 9) This is mainly due to the presence of an additional protein secretory component which in combination with two molecules of monomeric IgA results in a dimer that is more resistant to digestion by intestinal proteolytic enzymes (2) Although it is known that the majority of IgA in feeds of colostrum and milk is not absorbed by the infant (1 7 10) it has been suggested that the gut in early neonatal life is capable of absorbing small quantities of IgA (6 10) However the assay techniques which have been used have been relatively insensitive and it was considered desirable to examine this question using a very sensitive assay in a study of normally breast fed infants

The purpose of this study was to observe if exclusive breast feeding for 1 week produced a detectable effect on serum IgA levels in the neonate We have compared by means of a specific IgA radioimmunoassay the serum IgA concentrations in neonates after six days of exclusive breast feeding with serum IgA concentrations in neonates that were exclusively artificially fed In addition we have studied the metabolism of IgA in early life by analysing cord blood samples and comparing IgA concentrations present at birth with those found in the same infant six days later We have also estimated by gel filtration the molecular size of IgA in the neonate

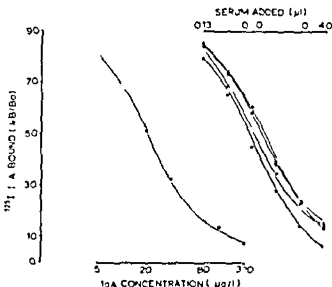


Fig 1 Inhibition curves for serum IgA standard (■—■) cord blood (▲—▲ and △—△) six day heel prick serum from an exclusively artificially fed neonate (○—○) and six day heel prick serum from an exclusively breast fed neonate (●—●)

MATERIALS AND METHODS

Sera

Forty eight healthy full term neonates were studied twenty three of the neonates were exclusively artificially fed and 25 were exclusively breast fed Cord blood was collected from 24

On the sixth day after delivery a heel prick blood sample is routinely taken for Guthrie testing and a further 0 5 ml blood sample was taken from the same site into a polythene capillary tube (Sarstedt) The blood was allowed to clot the serum separated by centrifugation and stored at −20°C till analysis

Quantitation of serum IgA concentrations

A preparation of human secretory IgA purified by the method of Newcomb (9) was kindly provided by Mr R R Samson This preparation was used for radioiodination and as a standard for gel filtration studies The serum IgA standard used (Hyland References Serum VI Lot 8559N005A) was standardised against the W H O International Standard The antiserum against serum IgA was raised in rabbits and was kindly provided by Dr T A E Platts Mills (11)

Radioiodination of secretory IgA

Iodine 125 labelled secretory IgA was prepared by the Chloramine T method (4) Forty µg of purified secretory IgA was added to 250 µCi of ^{125}I *in the presence of 50 µg Chloramine T* The reaction was terminated after 5 sec by the addition of 170 µg sodium metabisulphite The radioiodinated protein was separated from *inorganic* ^{125}I by gel filtration on Sephadex G 50 *(Pharmacia) the* ^{125}I IgA was further purified by Sepharose 4B (Pharmacia) gel filtration

Radioimmunoassay of IgA

Sodium phosphate buffer (0 05 mol/l) at pH 7 4 containing 2% horse serum (to reduce non specific binding) was used in making all dilutions and as the buffer and eluting medium in gel filtration and radioimmunoassay Serum IgA was measured by a double antibody radioimmunoassay 0 2 ml of a dilution of serum or of the serum IgA standard was added to 0 1 ml of anti IgA at 1/80 000 dilution and incubated at room temperature Three hours later 1 0 ng of ^{125}I IgA in 0 1 ml of buffer was added and the mixture incubated for a further 3 hours at room temperature Donkey anti rabbit serum (0 05 ml Wellcome) and non immune rabbit serum (0 05 ml Wellcome) were then added and the mixture incubated 16 hours at 4 C following which bound and free IgA was separated by centrifugation The antibody bound ^{125}IgA in the precipitate was measured in an automatic γ-counter (LKB) A standard curve was constructed for each assay by plotting the logit of the percent standard counts bound vs $\log_{10}$ ng of IgA added to the reaction mixture The IgA concentration of unknown serum samples was calculated from the standard inhibition curve The sensitivity of the radioimmunoassay procedure as defined by the concentration of IgA which produced a reduction of 10% in the specific binding of ^{125}I IgA tracer alone was 0 4 µg/l

Gel filtration

Gel filtration was carried out using Sepharose 4B (Pharmacia) in a 22×900 mm column with downward flow of eluate Three ml fractions were collected and stored at −20°C Immunoassay was performed on the unconcentrated fractions

Statistical methods

Student s *t* test was used to compare the arithmetic means of serum IgA concentrations in the breast fed and artificially fed neonates A paired Student s *t* test was used to compare cord blood samples with further serum samples taken from the same baby 6 days later

Reproducibility of the IgA assay

The intra assay coefficient of variation of the IgA RIA from 8 representative assays was 8 0% ($n=56$) The inter assay coefficient of variation from 9 representative assays was 8 1%

RESULTS

I Standard inhibition curve for IgA

The binding of ^{125}I secretory IgA to rabbit anti IgA was displaced in a parallel manner by the Hyland serum IgA standard, two samples of cord blood one sample of heel prick blood from a 6 day old neonate that had been artificially fed and one sample of heel prick blood from a 6-day old neonate that had been breast fed (Fig 1) This indicated that immunoassayable IgA was present in cord blood and day 6 heel prick blood

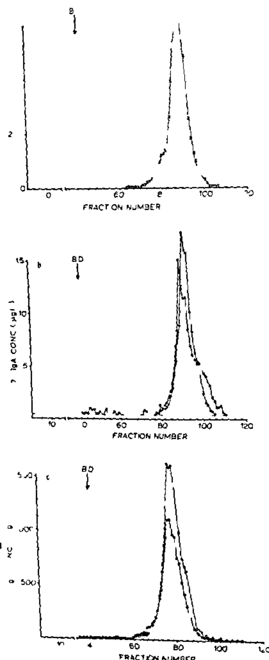


Fig 2 Gel filtration on Sepharose 4B column (? 2×90 cm) of (*a*) normal adult serum (×—×) (*b*) cord blood (○—○) pooled heel prick serum from 8 six-day-old neonates (4 artificially fed 4 breast fed) (●—●) and (*c*) colostrum (●—●) and milk (○—○) 3 ml fractions were collected and assayed by RIA for IgA using either the serum standard (serum samples) or the secretory standard (colostrum and milk) The arrow indicates the elution volume of a Blue Dextran marker (void volume) The broken line in (*b*) indicates the limit of detection in this

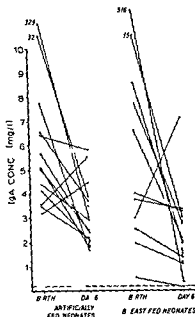


Fig 3 Serum IgA concentration in the cord blood of 24 newborn infants and heel prick sera of 23 exclusively artificially fed and 25 exclusively breast fed six-day-old neonates The solid lines indicate cord blood and heel prick sera that were collected from the same baby and the broken line indicates the limit of detection for serum IgA in this particular assay

2 *Characterisation of IgA in cord blood and day 6 serum*

A single peak of immunoassayable IgA on Sepharose 4B gel filtration was found in cord blood pooled sera from 4 artificially fed and 4 breast fed six day old neonates human colostrum human milk and from adult serum (Fig 2) Cord blood and heel prick serum IgA eluted at the same volume as adult serum IgA and at a different volume from secretory IgA present in milk and colostrum indicating that the predominant form of IgA in both cord blood and day 6 sera is the 7S monomeric form The trace of immunoassayable material found in infants serum at the elution volume of secretory IgA was at most 2 5% of the 7S peak suggesting that there is very little 11S IgA in day 6 serum The 7S IgA standard (Hyland) was therefore used for all subsequent measurements of IgA in cord blood and serum

3 *IgA concentration in cord blood*

The concentrations of IgA in 24 cord blood samples ranged from 0 52–516 0 mg/l Four neonates had cord blood levels that were greater than 10 mg/l and in two of these neonates IgA levels decreased from 516 mg/l to 0 33 mg/l and from 329 mg/l to 3 08 mg/l in 6 days (Fig 3)

A significant decrease in IgA concentrations occurred during the first 6 days of life in 20 neonates from whom both cord and day 6 sera were tested this was found in both artificially and breast fed neonates (paired *t* test $t=3\ 09$ $p<0\ 01$ Fig 3) The four neonates with cord blood levels greater than 10 mg/l were excluded from statistical analysis because their cord sera were probably contaminated with maternal blood

4 *IgA concentrations in day 6 serum*

Serum IgA levels in 23 exclusively artificially fed and 25 exclusively breast fed babies on the sixth day of life were 2 71±0 25 mg/l and 2 58±0 33 mg/l (arithmetic mean ± S E) respectively (Fig 3) There was no significant difference between mean IgA concentrations in the two groups showing that exclusive breast feeding had no detectable influence on serum IgA levels after 6 days One baby had less than 0 14 mg/l of IgA in its serum suggesting that it might be deficient in IgA This baby also had the lowest IgA concentration in its cord blood

DISCUSSION

We have shown on the basis of behaviour on gel filtration that IgA in the serum exists in a similar molecular form in both neonates and adults However IgA concentrations in the neonate are a thousand fold less than those in the adult In addition we have studied the influence of breast feeding on serum IgA concentrations and have failed to demonstrate an effect

The evidence for absorption of immunoglobulins from the neonatal gut is conflicting Amman & Stiehm (1) found no difference in immune globulin levels between artificially fed and breast fed infants after 4 days of breast feeding In contrast Iyengar & Selvaraj reported significantly higher immunoglobulins on the fifth day of life in colostrum fed infants compared with artificially fed infants (6) Ogra et al reported a rise in serum IgA levels in 3 infants who were fed colostrum 18 to 24 hours after birth suggesting that the gut is capable of absorbing IgA at this stage (10) In these studies IgA levels were determined by the relatively insensitive technique of single radial immunodiffusion (1 6) which in our experience has a lower limit of detection of 10 mg/l in addition Ogra et al used IgA poliovirus antibody as a marker for IgA in using the technique of radioimmunodiffusion (10) We have used a sensitive radioimmunoassay and low concentrations of IgA were found to be present in all neonates studied These levels were not influenced by breast feeding Furthermore we have shown that in infants sera less than 2 5% of the immunoreactive IgA like material behaves like 11 S IgA on gel chromatography Breast feeding would have been expected to produce an immunoreactive peak at the 11 S position in neonatal sera if the 11 S molecule was absorbed in the intact form by the neonatal gut

Our data demonstrates firstly that IgA absorption had ceased by the time of sampling at the sixth day and secondly that if there was a period of IgA absorption before the sixth day IgA entering the circulation must have been cleared with a half life value considerably less than the adult serum IgA half life value of approximately 5 days (16) We delayed collection of blood till the sixth day post partum because it was considered unethical to obtain samples earlier as no routine samples were being taken at that time

Our values for cord blood concentrations of IgA are consistent with the concentrations found by other workers using techniques of similar sensitivity (3 13) However we found

4 neonates with cord blood concentrations that were greater than 10 mg/l When these 4 neonates were studied 6 days later their respective serum IgA levels had decreased much more than would be expected if the half life of serum IgA in the infant is similar to that in the adult We therefore feel that these high values may be due to contamination of cord blood with maternal blood at the time of collection Earlier reports of raised IgA in cord blood serum (14) may therefore have been due to contamination of cord blood by maternal blood (with thousand fold higher IgA concentrations) at the time of collection

In the remaining 20 neonates in whom paired cord and day 6 sera were collected a significant decrease in serum IgA was observed in the first 6 days of life It is possible that this may have been due to transplacental transfer of maternal IgA or to minimal contamination with maternal blood at the time of cord blood collection We were unfortunately unable to distinguish between these two possibilities with the methodology available It is unlikely that this decrease is due to cord material interfering with a sensitive inhibition test as parallelism in an inhibition curve was observed between IgA in adult serum and IgA in cord blood In addition only a single peak of immunoreactivity (at the elution volume of adult serum IgA) was found in cord blood on examination by gel filtration Any substance that interfered with cord blood IgA determinations would have been expected to elute at a different volume on gel filtration to 7S IgA and therefore would have been detected on immunoassay of the gel filtration fractions

In one neonate we were unable to detect IgA at the assay dilution used indicating a level less than 0 14 mg/l This neonate also had the lowest cord blood value among all neonates studied Unfortunately we had insufficient samples for further analysis using more sensitive conditions for IgA detection but it is considered possible that this infant may be selectively deficient in IgA We are not aware that this condition has previously been detected in a neonate and follow up of this infant will be undertaken

In conclusion our data show that evidence of absorption of IgA into the circulation can not be detected in breast fed infants using a very sensitive assay It is likely that the principle physiological role of breast milk IgA in the infant must be sought at the level of the intestinal lumen or mucosal surfaces

ACKNOWLEDGEMENTS

We are grateful to Dr D G D Barr who helped us to obtain the blood samples to Dr T A E Platt Mills for the gift of antiserum for radioimmunoassay to Mr R R Samson for purified secretory IgA and to Dr W M Hunter for advice about radioimmunoassay One of us (P L Y) was supported by an M R C Clinical Training Fellowship during the period of the study

REFERENCES

1 Ammann A J & Stiehm E R Immune globulin levels in colostrum and breast milk and serum from formula and breast fed newborns *Proc Soc Exp Biol Med 122* 1098 1966

2 Brown W R Newcomb R W & Ishizaka K Proteolytic degradation of exocrine and serum immunoglobulin *J Clin Invest 49* 1374 1970

3 Faulkner W & Borella L Measurement of IgA levels in human cord serum by a new radioimmunoassay *J Immunol 105* 786 1970

4 Hunter W M & Greenwood F C Preparation of iodine 131 labelled human growth hormone of high specific activity *Nature (London) 194* 495 1962

5 Hurlimann J Waldesbuhl M & Zuber C Human salivary immunoglobulin A Some immunological and physicochemical characteristics *Biochim Biophys Acta 181* 393 1969

6 Iyengar L & Selvaraj R J Intestinal absorption of immunoglobulins by newborn infants *Arch Dis Child 47* 411 1972

7 Kenny J E Boesman M I & Michaels J G Bacterial and viral coproantibodies in breast fed infants *Pediatrics 34* 202 1967

8 McClelland D B L McGrath I & Samson R R Antimicrobial factors in human milk *Acta Paediat Scand 67* Suppl 271 1978

9 Newcomb R W Normansell D & Stanworth D R A structural study of human exocrine IgA globulin *J Immunol 101* 905 1968

10 Ogra S S Weingraub D & Ogra P L Immunologic aspects of human colostrum and milk *J Immunol 119* 245 1977

11 Platts Mills T A E & Ishizaka K IgG and IgA diphtheria antitoxin response from human tonsil lymphocytes *J Immunol 114* 1058 1975

1 Reddy V Bhaskaram C Raghuramulu N & Jaga

3 IgA concentration in cord blood

The concentrations of IgA in 24 cord blood samples ranged from 0 52–516 0 mg/l Four neonates had cord blood levels that were greater than 10 mg/l and in two of these neonates IgA levels decreased from 516 mg/l to 0 33 mg/l and from 329 mg/l to 3 08 mg/l in 6 days (Fig 3)

A significant decrease in IgA concentrations occurred during the first 6 days of life in 20 neonates from whom both cord and day 6 sera were tested this was found in both artificially and breast fed neonates (paired t test $t=3\ 09$ $p<0\ 01$ Fig 3) The four neonates with cord blood levels greater than 10 mg/l were excluded from statistical analysis because their cord sera were probably contaminated with maternal blood

4 IgA concentrations in day 6 serum

Serum IgA levels in 23 exclusively artificially fed and 25 exclusively breast fed babies on the sixth day of life were 2 71±0 25 mg/l and 2 58±0 33 mg/l (arithmetic mean ± S E) respectively (Fig 3) There was no significant difference between mean IgA concentrations in the two groups showing that exclusive breast feeding had no detectable influence on serum IgA levels after 6 days One baby had less than 0 14 mg/l of IgA in its serum suggesting that it might be deficient in IgA This baby also had the lowest IgA concentration in its cord blood

DISCUSSION

We have shown on the basis of behaviour on gel filtration that IgA in the serum exists in a similar molecular form in both neonates and adults However IgA concentrations in the neonate are a thousand fold less than those in the adult In addition we have studied the influence of breast feeding on serum IgA concentrations and have failed to demonstrate an effect

The evidence for absorption of immuno globulins from the neonatal gut is conflicting Amman & Stiehm (1) found no difference in immune globulin levels between artificially fed and breast fed infants after 4 days of breast feeding In contrast Iyengar & Selvaraj reported significantly higher immunoglobulins on the fifth day of life in colostrum fed infants compared with artificially fed infants (6) Ogra et al reported a rise in serum IgA levels in 3 infants who were fed colostrum 18 to 24 hours after birth suggesting that the gut is capable of absorbing IgA at this stage (10) In these studies IgA levels were determined by the relatively insensitive technique of single radial immunodiffusion (1 6) which in our experience has a lower limit of detection of 10 mg/l in addition Ogra et al used IgA poliovirus antibody as a marker for IgA in using the technique of radioimmunodiffusion (10) We have used a sensitive radioimmunoassay and low concentrations of IgA were found to be present in all neonates studied These levels were not influenced by breast feeding Furthermore we have shown that in infants sera less than 2 5% of the immunoreactive IgA like material behaves like 11 S IgA on gel chromatography Breast feeding would have been expected to produce an immunoreactive peak at the 11 S position in neonatal sera if the 11 S molecule was absorbed in the intact form by the neonatal gut

Our data demonstrates firstly that IgA absorption had ceased by the time of sampling at the sixth day and secondly that if there was a period of IgA absorption before the sixth day IgA entering the circulation must have been cleared with a half life value considerably less than the adult serum IgA half life value of approximately 5 days (16) We delayed collection of blood till the sixth day post partum because it was considered unethical to obtain samples earlier as no routine samples were being taken at that time

Our values for cord blood concentrations of IgA are consistent with the concentrations found by other workers using techniques of similar sensitivity (3 13) However we found

Acta Pædiatr Scand 68 701–704 1979

HYPERVISCOSITY OF THE BLOOD AND HAEMOSTASIS IN THE NEWBORN INFANT

P HENRIKSSON

From the Department of Paediatrics and Coagulation Laboratory University of Lund Allmanna Sjukhuset Malmo Sweden

ABSTRACT Henriksson P (Department of Paediatrics and Coagulation Laboratory University of Lund Allmanna Sjukhuset Malmo Sweden) Hyperviscosity of the blood and haemostasis in the newborn infant Acta Paediatr Scand 68 701 1979.—15 newborn infants with the hyperviscosity syndrome due to polycythaemia i e a central haematocrit of at least 65% and a raised whole blood viscosity were examined for changes in their coagulation and fibrinolytic systems 5 were thrombocytopenic but showed no other signs of activated coagulation Neither did the only patient with positive ethanol gelation test measuring circulating fibrin monomers Minute amounts of fibrin/fibrinogen degradation products (FDP) appeared in only two and with only one exception an assay for fibrinolytic activity in plasma was negative No defects were found in the coagulation system Thus in most of the patients there was no demonstrable abnormal proteolysis in the circulation However in such infants the normally low levels of antithrombin III (heparin cofactor activity) in combination with the impairment of the microcirculation might increase the risk of thrombotic complications Haemodilution preferably with plasma is therefore advocated in the symptomatic patients

KEY WORDS Polycythaemia hyperviscosity haemostasis newborn

Polycythaemia or hyperviscosity of the blood in the newborn accounts for a variety of symptoms of cardiorespiratory (5 9) cerebral (2 37) metabolic (15) and renal (1) nature Sporadic cases have been seen where hyperviscosity has been associated with neonatal complications such as necrotizing enterocolit (23) gangrene of a limb (31) thyrotoxicosis (4) and hydranencephaly (21) For review see Kontras (22) and Gross et al (11)

Coagulation changes associated with a high haematocrit have been described in 3 cases by Rivers (32) It would however appear that no other systematic studies of the haemostatic mechanism in such patients are available This paper concerns a detailed investigation of the coagulation and fibrinolytic systems in a group of 15 newborns with a central haematocrit of at least 65% and an increased viscosity of whole blood

CLINICAL MATERIAL

The material consisted of 15 newborns (7 boys and 8 girls) with a central haematocrit ranging from 65 to 73% (mean 68 4%) Their gestational age ranged from 35 to 42 weeks (mean 40 3) The lowest birthweight was 2250 g and the highest 5000 g (mean 3055 g) Placenta weight ranged from 290 to 760 g (mean 471 g) All the infants were plethoric Four were small for gestational age 5 had symptomatic hypoglycaemia 6 had respiratory symptoms one (the only preterm infant) had hyperbilirubinaemia and was treated with exchange transfusion at 33 h of age One had trisomy 21

Blood sampling Blood was collected through an umbilical vein catheter inserted for the purpose of haemodilution The blood sample was diluted in 1/10 volume of 3 8% citrate and plasma was treated as previously described (28) and stored at −70°C until examined The values were corrected for the actual haematocrit

METHODS

The platelets fibrinogen P&P (prothrombin factor VII and X) factor V factor VIII C were determined as described by Nilsson et al (27) and factor VIIIR Ag according to Holmberg & Nilsson (19) Fibrinolytic activity was measured on unheated fibrin plates as described by Nilsson & Olow (29) Plasminogen was determined immunochemically according to Ganrot & Nilehn (8) and fibrin/fibrinogen degradation products (FDP) were determined immunochemically according to Niléhn (26) on serum obtained from blood collected in tubes containing thrombin and EACA The ethanol gelation test of Godal et al (10) was used as a test for fibrin monomers Anti

deesan V Antimicrobial factors in human milk *Acta Paediatr Scand* 64 709 1977
13 Schuurman H J Hagenaars A M & Zegers B J M Cord serum levels of IgM IgA and Alpha fetoprotein and their inter relationships *Clin Chim Acta* 79 363 1977
14 Stiehm E R Ammann A J & Cherry J D Elevated cord macroglobulins in the diagnosis of intrauterine infections *N Engl J Med* 275 971 1966
15 Tomasi T B & Grey H M Structure and function of immunoglobulin A *Prog Allergy* 16 81 1972
16 Waldmann T A & Strober W Metabolism of immunoglobulins *Prog Allergy* 13 1 1969

Submitted Dec 5 1978
Accepted Febr 22 1979

(P L Y) Medical Research Council
Unit of Reproductive Biology
2 Forrest Road
Edinburgh EH1 2QW
Scotland

nected with high viscosity of the blood in the newborn can be ascribed to impairment of the flow of blood through small vessels particularly post capillary venules (25) Symptoms produced by the primary or parallel condition will add to this

Rivers (32) is the only author who has demonstrated and characterized coagulation changes in connection with a high haematocrit in the newborn Three infants with thrombocytopenia and a haematocrit above 65% were studied Using the serial protamine sulphate test (SDPST) as described by Gurewich & Hutchinson (13) he found circulating fibrin monomers indicating thrombin activity However no FDP were found This absence of co-existing FDP suggested low grade disseminated intravascular coagulation

In all but one of the present patients the ethanol gelation test was negative i e it did not reveal any fibrin monomers In a comparative study the ethanol gelation test proved equally sensitive as the SDPST while its specificity was debatable (14) Fibrin deposition within the vessels results in secondary fibrinolysis and FDP but practically no FDP were found Nor could we detect any decrease in those factors normally consumed in patients with intravascular coagulation namely fibrinogen prothrombin and to some extent factor VIII and AT III AT III was low in comparison with that in adults but at the same level as in healthy newborns (33) (Fig 3) The platelet counts were low in 5 but in none of them was the coagulation pattern consistent with activated coagulation Other possible explanations for the thrombocytopenia in these patients are impaired production due to tissue hypoxia (12) or to a secondary inhibition owing to a predominance of fetal erythropoiesis particularly during intrauterine growth retardation i e in infants small for gestational age The sluggish circulation also in the spleen might possibly increase the disappearance of platelets owing to their prolonged contact with the macrophages Furthermore in these patients with a small plasma volume in relation to the raised erythrocyte volume the platelet counts of whole blood will appear low while the concentration of platelets in plasma is normal

High levels of VIII C and VIIIR Ag are often seen in reactive processes and a discrepancy between them i e high VIIIR Ag in relation to VIII C may indicate pathological proteolysis in the circulation (17) VIII C and VIIIR Ag were slightly increased in our patients Only one showed a high VIIIR Ag/VIII C quotient but no other signs of existing coagulation or abnormal fibrinolysis

In conclusion we could not confirm that newborns with polycythaemia and hyperviscous blood have disturbances suggesting abnormal proteolysis in the blood However their physiologically low AT III levels (heparin cofactor activity) combined with a sluggish microcirculation are factors which might increase the risk of thrombotic complications impairing the tissue oxygenation still more

Haemodilution by venesection and replacement infusion preferably with plasma is indicated at least in the symptomatic patients (1 24 30)

ACKNOWLEDGEMENT

This investigation was supported by grants from the Swedish Medical Research Council (B79 19X-00087 15B) and the Medical Faculty University of Lund Sweden

REFERENCES

1 Aperia A Bergqvist G Broberger O Thodenius K & Zetterstrom R Renal function in newborn infants with high hematocrit values before and after isovolumetric haemodilution *Acta Paediatr Scand* 63 878 1974
2 Baum R S Hyperviscous blood and perinatal pathology *Pediatr Res* 1 ₄88 1967
3 Becker A H & Glass H Twin to-twin transfusion syndrome *Am J Dis Child* 106 6 4 1963
4 Bussman Y L Tillman M L & Pagliara A S Neonatal thyrotoxicosis associated with the hyper viscosity syndrome *J Pediatr* 90 266 1977
5 Danks D M & Stevens L H Neonatal respiratory distress associated with a high haematocrit reading *Lancet* II 499 1964
6 Ekelund H Hedner U & Nilsson I M Fibrinolysis in newborns *Acta Paediatr Scand* 59 33 1970

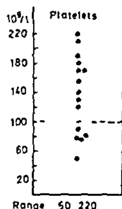


Fig 1 Platelet counts Dashed line denotes lower normal limit

thrombin III (AT III) was determined as described by Hedner & Nilsson (16) and α_2 macroglobulin (α_2M) according to Ganrot (7) The whole blood viscosity was measured at 37°C with a Wells Brookfield cone plate microviscometer (36) The haematocrit was measured in duplicate with a micro method

RESULTS

The results of the individual tests are given in the Figs 1–3 The ethanol gelation test was positive in 1 out of 13 patients examined FDP appeared in very low concentration in 2 out of 15 Fibrinolysis on fibrin plates was increased in 1 patient of 13 examined Only 5 cases had a moderate thrombocytopenia and in none of them were the levels of the coagulation factors low The whole blood viscosity was very high especially at slow shear rates (Fig 4)

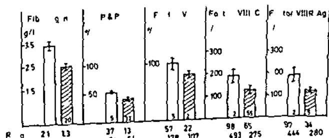


Fig 2 Mean standard error of the mean and range of fibrinogen P&P (prothrombin factor VII and factor X) factor V factor VIII C and factor VIIIR Ag Blank columns denote patients hatched columns healthy newborns according to Holmberg et al (18) Figures within the columns denote number of infants studied

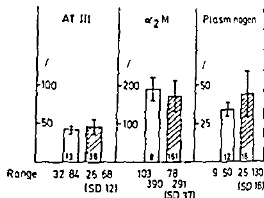


Fig 3 Mean standard error of the mean and range of antithrombin III α_2 macroglobulin and plasminogen Blank columns denote patients hatched columns healthy newborns according to Teger Nilsson (33) and Ekelund et al (6) Figures within the columns denote the number of infants studied

DISCUSSION

In the absence of maternal foetal or twin to-twin transfusion (3) or as a result of abnormal haemoglobin (34) the etiology of polycythaemia in the newborn is obscure It is often associated with small for gestational age infants (20) or with syndromes such as trisomy 21 (35) The most heterogenous symptoms con

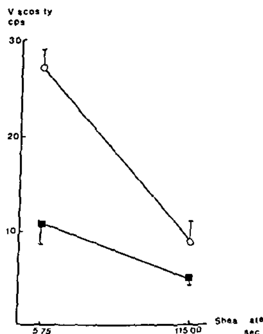


Fig 4 Mean and standard deviation of whole blood viscosity in eight patients (O) and in 12 healthy adults (■) at shear rates of 115 sec and 5 75 sec⁻¹ respectively

Acta Pædiatr Scand 68 705–708 1979

MEDICINAL IRON TO LOW BIRTH WEIGHT INFANTS

L. JANSSON L. HOLMBERG and R. EKMAN

From the Department of Paediatrics University of Lund Malmo General Hospital Malmo and the Department of Clinical Chemistry University Hospital Lund Sweden

ABSTRACT Jansson L Holmberg L and Ekman R (Department of Paediatrics University of Lund Malmo General Hospital Malmo Sweden and Department of Clinical Chemistry University Hospital Lund Sweden) Medicinal iron to low birth weight infants Acta Paediatr Scand 68 705 1979 —*Serum ferritin concentrations were measured during the first 6 months of life in 28 low birth weight infants (mean birth weight 1820 g range 900–2460 mean gestational age 34 weeks range 29–37) fed a standard formula fortified with ferrous sulphate Fifteen of the infants received supplementary medicinal iron (ferrous succinate) from 3 weeks of age and 13 only from 2 months of age All were given vitamin E from 10 days of age The serum ferritin values did not differ between the groups at 1–2 days 8–10 weeks or at 6 months Furthermore there were no signs of hyperhaemolysis at 8–10 weeks in the group receiving medicinal iron early The data indicate that the iron content in the formula is sufficient until 2 months of age but also that there is no disadvantage in starting medicinal iron at 3 weeks of age if the diet is sufficient in vitamin E.*

KEY WORDS S ferritin low birth weight medicinal iron vitamin E

The foetus accumulates iron at a rate proportional to the increase in body weight (6) Consequently the low birth *weight (LBW) infant* is at risk of developing iron deficiency and iron supplementation is recommended (2) The iron can be given either as a formula fortification or as a medicinal *iron preparation* The infant formulas produced in Sweden are fortified with either a ferric or a ferrous salt Because of the poor iron absorption from the ferric salts (11) it has hitherto been the rule to give a medicinal iron preparation (ferrous sulphate or ferrous succinate) in addition to the iron from the formula

Iron supplementation to LBW infants is however not without risks Because of relative vitamin E deficiency in these infants (9) iron supplementation may increase red cell haemolysis and thus aggravate the physiological anaemia The purpose of this study was two-fold First to determine the haematological effects of medicinal iron supplementation in LBW infants fed a standard formula fortified with ferr[illegible]phate and secondly to assess the relationship between iron intake and the iron stores in LBW infants during the first 6 months of age The iron stores of the infants were estimated with the serum ferritin assay which under basal conditions has proved to be a simple and quantitative method for determining iron stores (3 4 7 13 16)

PATIENTS AND METHODS

The material consisted of 31 LBW infants with a birth weight (BW) ≤2000 g and/or a gestational age of ≤35 weeks All infants were born in Malmo General Hospital between December 1975 and May 1977 *Infants with* haemoglobin values below 150 g/l or above 260 g/l were not accepted neither were those with signs of haemolysis or those requiring blood transfusions Two infants with mild respiratory distress were accepted Three infants were subsequently excluded because of a different feeding regimen Of the remaining 28 infants none showed any sign of hepatic or renal disease The gestational age of each infant was assessed from a combination of maternal data external characteristics (15) and neurological evaluation (1) The mean birth weight was 1820 g (range 900–2460) and the mean gestational age was 34 weeks (range 29–37)

The infants were randomly distributed among two therapeutic regimens

7 Ganrot P O Determination of α_2 macroglobulin as trypsinprotein esterase *Clin Chim Acta 14* 493 1966
8 Ganrot P O & Niléhn J E Immunochemical determination of human plasminogen *Clin Chim Acta 22* 335 1968
9 Gatti R A Muster A J Cole R B & Paul M H Neonatal polycythemia with transient cyanosis and cardiorespiratory abnormalities *J Pediatr 69* 1063 1966
10 Godal H C & Abildgaard U Gelation of soluble fibrin in plasma by ethanol *Scand J Haematol 3* 342 1966
11 Gross G P Hathaway W E & McGaughey H R Hyperviscosity in the neonate *J Pediatr 82* 1004 1973
12 Gross S Keefer V & Liebman J The platelets in cyanotic congenital heart disease *Pediatrics 42* 651 1968
13 Gurewich V & Hutchinson E Detection of intravascular coagulation by a serial dilution protamine sulfate test *Ann Intern Med 75* 895 1971
14 Gurewich V Lipinski B & Lipinska I A comparative study of precipitation by protamine sulfate and ethanol gelation tests *Thromb Res 2* 539 1973
15 Haworth J C Dilling L & Younoszai M K Relation of blood glucose to hematocrit birthweight and other body measurements in normal and growth retarded newborn infants *Lancet II* 907 1967
16 Hedner U & Nilsson I M Antithrombin III in a clinical material *Thromb Res 3* 631 1973
17 Henriksson P & Holmberg L Factor VIII activity and antigen in sick newborns with pathological proteolysis in blood *Acta Paediatr Scand 67* 83 1978
18 Holmberg L Henriksson P Ekelund H & Åstedt B Coagulation in the human fetus *J Pediatr 85* 860 1974
19 Holmberg L & Nilsson I M Immunologic studies in haemophilia A *Scand J Haematol 10* 12 1973
20 Humbert J R Abelson H Hathaway W E & Battaglia F C Polycythemia in small for gestational age infants *J Pediatr 75* 812 1969
21 Koffler H Keenan W J & Sutherland J M Hydranencephaly following elevated hematocrit values in a newly born infant *Pediatrics 54* 770 1974
22 Kontras S Polycythemia and hyperviscosity syndromes in infants and children *Pediatr Clin North Am 19* 919 1972
23 Leake R D Thanopoulos B & Nieberg R Hyperviscosity syndrome associated with necrotizing enterocolitis *Am J Dis Child 129* 1192 1975
24 Mackintosh T F & Walker C H M Blood viscosity in the newborn *Arch Dis Child 48* 547 1973
25 Messmer K Hemodilution *Surg Clin North Am 55* 659 1975
26 Nilehn J E Separation and estimation of split products of fibrinogen and fibrin in human serum *Thromb Diath Haemorrh 18* 487 1967
27 Nilsson I M Blomback M & Ramgren O Haemophilia in Sweden I Coagulation studies *Acta Med Scand 170* 665 1961
28 Nilsson I M Blomback M & von Francken I On an inherited autosomal haemorrhagic diathesis with antihaemophilic globulin (AHG) deficiency and prolonged bleeding time *Acta Med Scand 159* 35 1957
29 Nilsson I M & Olow B Determination of fibrinogen and fibrinogenolytic activity *Thromb Diath Haemorrh 8* 297 1962
30 Oski F A & Naiman J L Hematologic problems in the newborn W B Saunders & Co Philadelphia 1972 pp 78–82
31 Papageorgiou A & Stern L Polycythemia and gangrene of an extremity in a newborn infant *J Pediatr 81* 985 1972
32 Rivers R P A Coagulation changes associated with a high haematocrit in the newborn infant *Acta Paediatr Scand 64* 449 1975
33 Teger Nilsson A C Antithrombin in infancy and childhood *Acta Paediatr Scand 64* 624 1975
34 Weatherall D J Polycythemia resulting from abnormal hemoglobin *N Engl J Med 280* 604 1969
35 Weinberger M M & Oleinick A Congenital marrow dysfunction in Down s syndrome *J Pediatr 77* 273 1970
36 Wells R E Jr Denton R & Merrill D W Measurement of viscosity of biologic fluids by cone plate viscometer *J Lab Clin Med 57* 646 1961
37 Wood J L Plethora in the newborn associated with cyanosis and convulsions *J Pediatr 64* 143 1959

Submitted Febr 7 1979
Accepted March 20 1979

(P H) Department of Paediatrics
Allmänna Sjukhuset
S 214 01 Malmö
Sweden

Table 2 *Haematological status at 8–10 weeks of age*

Mean values (±1 S D)

Therapy group	No	Haemoglobin (g/l)	Reticulocytes (×10 /l)	Platelets $(\times 10^9/l)$
A	15	103 (10)	60 (30)	383 (171)
B	13	97 (10)	47 (27)	453 (105)

The haematological data at 8–10 weeks and at 6 months of age are given in Table 2 and 3 The groups did not differ significantly from one another in haemoglobin concentration reticulocyte and platelet counts

DISCUSSION

In the present study we have investigated the effects of early versus late medicinal iron supplementation to LBW infants Owing to a low level of s tocopherol LBW infants are sensitive to oxidant stress from dietary factors The most important of such factors are supplemental iron and polyunsaturated fatty acids Even a moderate dose of iron (2 mg/kg/day) increases red cell haemolysis if the diet is rich in polyunsaturated fat (32 4% of the fat as linoleic acid) (17) The infants examined by us were fed a formula in which linoleic acid constituted 17% of the fat In addition to the tocopherol content of the formula (8 mg/l) the infants received supplemental tocopherol acetate (16 5 mg/day) This amount gives a satisfactory level of serum α tocopherol (5) Under these feeding conditions we found no signs of increased haemolysis (low Hb high reticulocyte count) in the group receiving supplemental medicinal iron already from 3 weeks of age All the infants in our study were fed a formula fortified with 10 mg iron/l as ferrous sulphate which corresponds to an iron intake of nearly 2 mg/kg/day This dose has earlier been found to prevent iron deficiency in LBW infants (4) i e when the iron is present as an readily available salt According to Rios et al (11) fortification with ferric salts such as ferric orthophosphate and ferric pyrophosphate is not sufficient to meet the needs of the infant Nevertheless formulas fortified with ferric salts are still on sale in Sweden

In the infants studied by us we found no difference in s ferritin at two months of age whether the infants had received supplemental medicinal iron from 3 weeks of age or not However s ferritin values may be difficult to evaluate when supplemental iron is given as iron therapy can by itself raise s ferritin in iron deficiency before the iron stores are fully saturated (13 14) The effect of the dose of supplemental iron on s ferritin has not been fully evaluated but as s ferritin showed the same development during the first six months in the two groups there seems to be no advantage with the early medicinal iron supplementation

When investigating the requirement of iron supplementation to LBW infants the length of breast feeding should also be considered In our study only two of the mothers were able to breast feed their infants for 6 weeks The value of prolonged breast feeding in protecting term infants from iron deficiency has been stressed by Saarinen et al (12) In LBW infants however Lundstrom et al (8) found no differences in s ferritin and Hb at 3 months of age whether the infants were fed breast milk

Table 3 *Haematological status at 6 months of age*

Mean values (±1 S D)

Therapy group	No	Haemoglobin (g/l)	Reticulocytes $(\times 10^9/l)$
A	10	115 (7)	20 (15)
B	9	115 (5)	15 (8)

Table 1 *Birth weight and haematological status at birth in the two therapy groups*
Mean values (±1 S D)

Therapy group	No	Birth weight (gram)	Haemoglobin (g/l)	Reticulocytes (×10⁹/l)	Platelets (×10 /l)
A	15	1 855 (430)	200 (27)	135 (75)	140 (71)
B	13	1 779 (327)	191 (29)	166 (75)	160 (73)

(A) Ferrous succinate 2-3 mg/kg/day (Ferromyn S® AB Hassle) from 3 weeks of age

(B) Ferrous succinate 2-3 mg/kg/day from 2 months of age

All the infants were given the following vitamin preparations from 10 days of age 1/2 ml *Protovit® (Roche)* supplying vitamin A C D the B complex and 1 5 mg tocopherol acetate/day tetrahydrofolic acid 50 μg/day (Leucovorin® Lederle) and 15 mg tocopherol acetate/day (E vitamin® AB ACO)

Feeding routines All the infants were given human pasteurized milk for a period of at least 2 weeks or until they weighed 2 100 g If the breast milk supply by the mothers was then insufficient to satisfy the infants requirement a commercial standard formula Milkotal (AB Findus) was given Milkotal contains 8 mg tocopherol acetate/l and linoleic acid accounts for 17% of the total fat The formula is fortified with 10 mg ferrous sulphate/l After the discharge from hospital two infants were partly breast fed until 6 weeks of age Solid food and cereals were introduced at 4–5 months of age

The infants were examined haematologically within the first 24 hours at 8–10 weeks and at 6 months of age Blood for determining the serum ferritin concentration was taken at 24–48 hours at 8–10 weeks and at 6 months of age All blood samples were obtained by heel puncture sera were stored at −70°C until analysed

S ferritin was determined according to the method of Miles et al (10) with reagents from Ramco Laboratories Inc Houston Texas The statistical analyses were calculated with Student s *t* test In the case of s ferritin the statistical analyses were performed after log transformation (3 4 7 13 16)

RESULTS

The mean birth weights and the haematological data at birth in the groups are given in Tabel 1 The two groups were similar in birth weight haemoglobin concentration reticulocyte and platelet counts

Fig 1 shows the s ferritin concentration in the two groups at 1–2 days 8–10 weeks and at 6 months of age In group A (medicinal iron from 3 weeks) the mean s ferritin fell from 102 μg/l (range 34–220) at 1–2 days to 56 μg/l (range 16–175) at 8–10 weeks and to 26 μg/l (range 18–45) at 6 months of age In group B (medicinal iron from 2 months) we found a similar fall in s ferritin from 100 μg/l (range 45–200) at 1–2 days to 72 μg/l (range 21–170) at 8–10 weeks of age to 28 μg/l (range 10–115) at 6 months of age S ferritin did not differ between the groups on any of these occasions This was also true when only those of the infants with a birth weight of ≤2 000 g were taken into account In these infants the mean s ferritin at 8–10 weeks in group A (11 infants) was 27 (range 16–116) μg/l and in group B (11 infants) 47 (range 21–170) μg/l

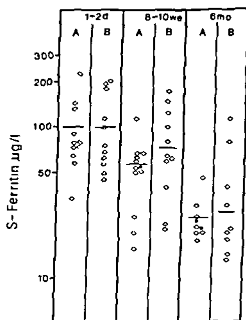


Fig 1 S ferritin concentration in 2 groups of LBW infants at 1–2 days 8–10 weeks and at 6 months of age (*A*) Ferrous succinate from 3 weeks (*B*) Ferrous succinate from 2 months The bars in the figure denote geometrical means ☐ infants with BW ≤2 000 g ● infants with BW >2 000 g

Table 2 *Haematological status at 8–10 weeks of age*

Mean values (±1 S D)

Therapy group	No	Haemoglobin (g/l)	Reticulocytes (×10 /l)	Platelets (×10⁹/l)
A	15	103 (10)	60 (30)	383 (171)
B	13	97 (10)	47 (?7)	453 (105)

The haematological data at 8–10 weeks and at 6 months of age are given in Table 2 and 3 The groups did not differ significantly from one another in haemoglobin concentration reticulocyte and platelet counts

DISCUSSION

In the present study we have investigated the effects of early versus late medicinal iron supplementation to LBW infants Owing to a low level of s tocopherol LBW infants are sensitive to oxidant stress from dietary factors The most important of such factors are supplemental iron and polyunsaturated fatty acids Even a moderate dose of iron (2 mg/kg/day) increases red cell haemolysis if the diet is rich in polyunsaturated fat (32 4% of the fat as linoleic acid) (17) The infants examined by us were fed a *formula in which linoleic acid* constituted 17% of the fat In addition to the tocopherol content of the formula (8 mg/l) the infants received supplemental tocopherol acetate (16 5 mg/day) This amount gives a satisfactory level of serum α tocopherol (5) Under these feeding conditions we found no signs of increased haemolysis (low Hb high reticulocyte count) in the group receiving supplemental medicinal iron already from 3 weeks of age All the infants in our study were fed a formula fortified with 10 mg iron/l as ferrous sulphate which corresponds to an iron intake of nearly 2 mg/kg/day This dose has earlier been found to prevent iron deficiency in LBW infants (4) i e when the iron is present as an readily available salt According to Rios et al (11) fortification with ferric salts such as ferric orthophosphate and ferric pyrophosphate is not sufficient to meet the needs of the infant Nevertheless formulas fortified with ferric salts are still on sale in Sweden

In the infants studied by us we found no difference in s ferritin at *two months of age* whether the infants had received supplemental medicinal iron from 3 weeks of age or not However s ferritin values may be difficult to evaluate when supplemental iron is given as iron therapy can by itself raise s ferritin in iron deficiency before the iron stores are fully saturated (13 14) The effect of the dose of supplemental iron on s ferritin has not been fully evaluated but as s ferritin showed the same development during the first six months in the two groups there seems to be no advantage with the early medicinal iron supplementation

When investigating the requirement of iron supplementation to LBW infants the length of breast feeding should also be considered In our study only two of the mothers were able to breast feed their infants for 6 weeks The value of prolonged breast feeding in protecting term infants from iron deficiency has been stressed by Saarinen et al (12) In LBW infants however Lundstrom et al (8) found no differences in s ferritin and Hb at 3 months of age whether the infants were fed breast milk

Table 3 *Haematological status at 6 months of age*

Mean values (±1 S D)

Therapy group	No	Haemoglobin (g/l)	Reticulocytes (×10⁹/l)
A	10	115 (7)	0 (15)
B	9	113 (5)	15 (8)

or a formula not fortified with iron Their findings suggest that an iron supplement should be given also to breast fed LBW infants

REFERENCES

1 Amiel Tisson C Neurological evaluation of the maturity of newborn infants *Arch Dis Child 43* 89 1968
2 Committee on Nutrition Iron supplementation for infants *Pediatrics 58* 765 1976
3 Cook C D Lipschitz D A Miles L E M & Finch C A Serum ferritin as a measure of iron stores in normal subjects *Am J Clin Nutr 27* 681 1974
4 *Jacobs A Miller F Worwood M Beamish M R* & Wardrop C A Ferritin in the serum of normal subjects and patients with iron defincienc) and iron overload *Br Med J IV* 206 1972
5 Jansson L Holmberg L Nilsson B & Johansson B Vitamin E requirements of preterm infants *Acta Paediatr Scand 67 459 1978*
6 Lanzkovsky O Iron metabolism in the newborn infant *Clin Endocrinol Metabol 5* 149 1976
7 Lipschitz D A Cook J D & Finch C A A clinical evaluation of serum ferritin as an index of iron stores *N Engl J Med 209* 1213 1974
8 Lundstrom U Siimes M A & Dallman P R At what age does iron supplementation become necessary in low birth weight infants? *J Pediatr 91* 878 1977
9 Melhorn D K & Gross S Vitamin E dependent anaemia in the premature infant I Effects of large doses of medicinal iron *J Pediatr 79* 560 1971
10 Miles L E M Lipschitz D A Bieber C P & Cook J D *Measurement of serum ferritin by a 2 site* immunoradiometric assay *Anal Biochem 61* 09 1974
11 Rios E *Hunter* R E Cook J D Smith N J & Finch C A The absorption of iron as supplement in infant cereal and infant formulas *Pediatrics 5?* 686 1975
12 Saarinen U M Siimes M A & Dallman P R Iron absorption in infants High bioavailability of breast milk iron is indicated by the extrinsic tag method *of iron absorption and by the concentration of* serum ferritin *J Peadiatr 91* 36 1977
13 Siimes M A Addiego J E & Dallman P R Ferritin in serum Diagnosis of iron deficiency and iron overload in infants and children *Blood 43* 581 1974
14 Thomas W J Koenig H M Lightsey A L Jr *& Green R* Free *erythrocyte porphyrin* Hemoglobin ratios serum ferritin and *transferrin* saturation levels during treatment of infants with iron deficiency anaemia *Blood 49* 455 1977
15 Usher R McLean F & Scott K Judgement of foetal age II Clinical significans of gestational age and an objective method for its assessment *Pediatr Clin North Am 13 835* 1966
16 Walters G O Miller F M & Worwood M Serum ferritin concentration and iron stores in normal subjects *J Clin Pathol 26* 770 1973
17 Williams M L Shott R J O Neal P L & Oski F A Role of dietary iron and fat on vitamin E deficiency of infancy *N Engl J Med* 29? 887 1975

Submitted Dec 11 1978
Accepted March 7 1979

(L J) Department of Paediatrics
Malmo Allmanna Sjukhus
S 21401 Malmö
Sweden

THE NUMBER OF POLYMORPHONUCLEAR LEUKOCYTES IN RELATION TO GESTATIONAL AGE IN THE NEWBORN

L COULOMBEL M DEHAN G TCHERNIA
C HILL and M VIAL

From the Laboratoire Central d Hematologie and the Service de Reanimation Neonatale Hopital Antoine Beclere Clamart France

ABSTRACT Coulombel L Dehan M Tchernia G Hill C and Vial M (Laboratoire Central d Hematologie and Service de reanimation neonatale Hopital Antoine Beclere Clamart France) The number of polymorphonuclear leukocytes in relation to gestational and post natal ages in non infected distressed newborns Acta Paediatr Scand 68 709 1979 —173 distressed newborn infants without evidence of bacterial infection were investigated at 12 hour intervals for the number of polymorphonuclear leukocytes during the first 5 days of life The results showed a significant difference in the number of polymorphonuclear leukocytes in relation to both gestational and post natal ages The study stresses the necessity of taking into account these differences in interpreting neutropenia as a sign of bacterial infection in neonates

KEY WORDS Newborn infants bacterial infection neutropenia

Within the first days of life the existence of neutropenia is usually considered a sign of serious bacterial infection (1 2 5 6) In an attempt to define numerical limits on the definition of neutropenia we studied in a population of distressed but non infected newborns the variations in the number of polymorphonuclear leukocytes (PMN) in relation both to gestational and post natal ages

MATERIAL AND METHODS

All newborn infants of age less than 5 days admitted to the neonatal intensive care unit during a nine month period were included in this study All these infants had some kind of distress and none of them could be considered as normal infants 70% of all blood samples and 85% of the first 48 hours samples were obtained by umbilical catheters Other samples were obtained by heel pricks The variation of hemoglobin values in the different samples drawn at 1 hour intervals did not exceed 15 g/l Thus we assumed that all WBC counts could be taken in account and compared even when the sampling technique was different Total leukocyte enumeration was performed by a Coulter S blood cell counter and was verified by hemocytometer if the white blood cell count was less than 3000/mm³ A differential count was performed by classifying 200 nucleated cells on a smear stained with May Grunwald-Giemsa

These observations were initiated when the infant entered the unit were repeated every 12 hours up to 48 hours of age and were continued every 24 hours afterwards through the 4th day of life 240 newborn infants were included in this investigation 67 were excluded from the statistical analysis either because bacterial infection was verified (15 infants) or because infection was thought probable but not proven by bacteriological results (52 infants) The remaining 173 infants without bacterial infection were retained for the statistical analysis They were subdivided into 3 groups of different gestational age Namely group 1 37 weeks or more group 2 33 to 36 weeks and group 3 32 weeks or less Among these 173 newborns samples during the first 12 hours of life were obtained for 132 infants For the time intervals after the first 12 hours we included data *from* all available patients even if the early blood samples had not been obtained

RESULTS

As shown in Fig 1 within the first twelve hours of life in 132 newborns a correlation appeared between gestational age and the number of PMN/mm³ as gestational age *progressed* the mean number of PMN/mm³ progressively increased During this initial 12 hour period the mean ±2 S E M number of

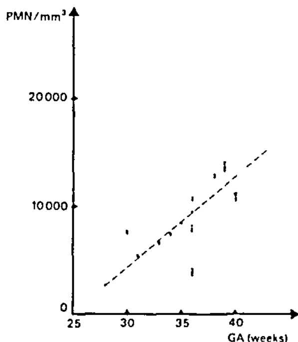


Fig 1 PMN/mm³ in relation to gestational age in 132 newborns within the first 12 hours of life The formula for the equation of the regression line allowing calculation of the number of PMN in relation to gestational age is PMN×GA 16700 $r=0.52$ $p<0.001$

PMN was 12000±1400/mm³ in 58 infants of group 1 8000±1300 in 49 infants of group 2 and 6000±1000/mm³ in 25 infants of group 3 The evolution of the number of PMN/mm³ as compared in the 3 gestational age groups appears in Fig 2

The variation of PMN after birth was similar in the 3 groups of infants After an initial increase between 12 and 24 hours of life there was a decrease in all groups that was more pronounced in group 1 than in groups 2 and 3 between the 24th and 72nd hour of life This greater decrease in group 1 resulted in similar levels of PMN in all 3 groups by the 4th day (Fig 2)

DISCUSSION

During fetal life hematopoiesis occurs early in the yolk sac during the third gestational week Then fetal hematopoietic cells migrate successively to different sites primarily to the fetal liver and by the 4th month to the bone marrow (4) At this stage there is a major commitment to erythroid differentiation but a proportion of cells have been shown to be capable of granulocyte differentiation in other environments as shown by Moore & Williams (3) In vivo this granulocytic differentiation mainly occurs in bone marrow during the last gestational weeks and gradually increases This process can explain the correlation between the number of PMN and the gestational age The loss of this correlation during the first week after birth suggests that natal or postnatal events lead to a regulation of the granulocyte population which eliminate the differences in children of various gestational ages by the end of the 1st week of life

This fact to our knowledge had not been found in previous studies In sick newborn infants early detection and prompt treatment of bacterial infection is necessary to avoid a fulminant course of disease leading frequently to death Since neutropenia has been shown to represent a useful and early sign of severe bacterial infections it appears important to define the limits of this criterion Our results emphasize the necessity of referring to both gestational and postnatal ages in interpreting the significance of the number of PMN in sick newborns For example (Fig 2) 5000 PMN/mm³ at less than 12 hours of age is a normal figure for an infant born at 32 weeks of gestation but is pathologic for a term neonate of the same postnatal age

At present these figures are employed routinely in our intensive care unit and are useful in the diagnosis of neonatal infection

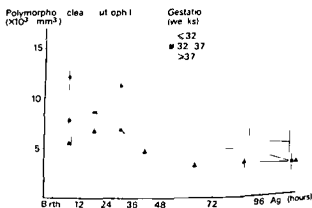


Fig 2 Evolution of PMN/mm³ as compared in the 3 gestational groups

ACKNOWLEDGEMENTS

This study has been granted by the Caisse Regionale d Assurance Maladie de l'Ile de France

REFERENCES

1 Gregory J & Hey E Blood neutrophil response to bacterial infection in the first month of life *Arch Dis Child 47* 747 197

Kuchler H Fricker H & Gugler E La formule sanguine dans le diagnostic precoce de la septicemie du nouveau né *Helv Paediatr Acta 31* 33 1976

3 Moore M A S & Williams N Analysis of proliferation and differentiation of fetal granulocyte macrophage progenitor cells in haemopoietic tissue *Cell Tissue Kinet 6* 461 1973

4 Oski F A & Naiman J L *Hematologic problems in the ne b rn* W B Saunders Company Philadelphia 1972 2nd ed p 377

5 Xanthou M Leucocyte blood picture in ill newborn babies *Arch Dis Child 47* 741 1972

6 Zipurski A & Jaber H M The haematology of bacterial infection in newborn infants *Clin Haematol* 7 175 1978

Submitted July 28 1978
Accepted Febr 5 1979

(G T) Laboratoire Central d Hématologie
Hopital Antoine Beclere
157 Rue de la Porte de Trivaux
92141 Clamart
France

Acta Pædiatr Scand 68 713–718 1979

UMBILICAL ARTERY CATHETERIZATION IN NEWBORNS

II Infections in Relation to Catheterization

GORAN WESSTRÖM and ORVAR FINNSTRÖM

From the Department of Paediatrics University Hospital Linkoping Sweden

ABSTRACT Wesstrom G and Finnstrom O (Department of Paediatrics University Hospital Linkoping Sweden) Umbilical artery catheterization in newborns II Infections in relation to catheterization Acta Paediatr Scand 68 713 1979.—Infections as a complication of umbilical artery catheterization were studied in 65 sick newborn infants Every second day during the catheterization period peripheral blood cultures as well as blood samples for white cell count and platelets were taken Cultures were taken from the catheter tips and from the umbilicus at the time of withdrawal of the catheter Local or systemic antibiotics were not used prophylactically but on rather broad clinical indications No case of septicemia was found but 8 infants had positive blood cultures and 16 had positive catheter tip cultures Positive catheter tip culture occurred more often in infants who were born before 32 weeks of gestational age Neither the duration of the catheterization nor the treatment with antibiotics influenced significantly the frequency of positive cultures

KEY WORDS Newborn infant umbilical artery catheterization infection

Umbilical artery catheterization is an accepted procedure for obtaining arterial acid base values in sick newborn infants During the almost 20 years in which this method has been used various complications have been described such as thrombosis/embolism vascular perforation ischemic necrosis of abdominal viscera hemorrhage blanching of the legs and infection These have been reviewed in an earlier paper (31)

A few prospective studies have evaluated the risk of local or systemic infection associated with umbilical artery catheterization The frequency of septicemia in these studies varied between 0 and 8% (4 12 26 29) and the frequency of bacterial colonization of the catheters between 6 and 55% (8 22 25)

The value of local and/or systemic antibiotics to prevent infections at umbilical catheterization has been investigated with varying results (3 4 5 22 24 29)

The present study was undertaken to analyze the following problems in connection with arterial catheterization

- the risk of systemic infection
- the significance of positive catheter tip and blood cultures
- the value of antibiotics in the prevention of infection
- the correlation between certain clinical parameters and the risk for systemic infection

MATERIAL

During a 19 month period 71 sick newborn infants had an umbilical artery catheter introduced Six infants were excluded from the study 3 because of early death before cultures had been taken and 3 for other reasons The indications for catheterization have been given in an earlier paper (31) Fourty-seven infants had a low birth weight (≤2500 g) 54 were preterm (≤37 weeks of gestational age) 17 had severe postnatal asphyxia (Apgar ≤6 at 5 min) in 13 there was an early rupture of the membranes (>24 hours) and 24 experienced a complicated delivery such as breech presentation or caesarean section on foetal indication

Fifty three infants were fed with human milk In 46 the feeding was started during the first 3 days of life and in 7 after the 3rd day but during the catheterization period The remaining 12 *infants did not* receive human milk 7 because of severe illness or early death and 5 because no human milk was available The human milk was stored

Table 1 *Clinical data of the 65 infants*

	Mean	S D	Range
Birth weight (g)	2 103	799	960–4 670
Gestational age (weeks)	34 7	3 5	26–43
Apgar score at 5 min	7 6	2 3	1–10
Duration of catheterization (hours)	69 3	40 1	6–173
Degree of illness (score 1–5)	2 5	1 0	1–5

frozen thawed and rapidly heated to about 95 C and then cooled in a water bath before use

Blood cultures were performed in 61 infants catheter tip cultures in 58 and umbilical cultures in 53 All types of cultures could not be done in all infants

The mean values and range for birth weight gestational age Apgar score at 5 min duration of catheterization and degree of illness are given in Table 1 Degree of illness was subjectively estimated according to a 5 degree scale where score 5 consisted of the infants who died score 4 and 3 of severely sick infants as exemplified by the need for mechanical ventilation or continuous positive airway pressure (CPAP) and score 2 and 1 the remaining less sick infants

METHODS

The catheterization was performed with a strictly aseptic technique The umbilical stump and the surrounding skin were cleaned with 0 5% Chlorhexidine alcohol Mainly for the purpose of studying the frequency of thromboses (31) two types of catheters Argyle umbilical artery and Argyle feeding tube were used and inserted in the aorta either short (L_{3-5}) or long (Th_{8-11}) After insertion of the catheter the umbilicus as well as the stopcock were covered with a sterile dressing The stopcock and supplying connections were changed daily There was no topical application of antibiotics The catheter was used both for blood sampling and for infusion When drawing blood sterile gloves were worn A 10% glucose solution was infused in all infants If necessary blood transfusions or other fluids e g sodium bicarbonate were given through the catheter

Table 2 *Indications for treatment with antibiotics in 22 infants*

	No of infants
Intrauterine infection suspected	5
Postnatal septicemia suspected	9
Pneumonia suspected	5
Positive blood culture	2
Necrotizing enterocolitis	1
Total	22

Table 3 *Number of positive cultures and the types of the bacteria*

In 3 instances more than one type of bacteria was found in the culture

	Blood culture	Catheter tip culture	Umbilical culture
Gram positive bacteria			
Staphylococcus epidermidis	6	1	8
Staphylococcus aureus		5	6
Beta hemolytic streptococcus		4	1
Enterococcus		1	
Alpha streptococcus			2
Gamma streptococcus			1
Diphtheroids	1		
Gram negative bacteria			
Escherichia coli	1	2	2
Klebsiella/Enterobacter		2	2
Proteus		1	
Pseudomonas		1	
Coliforms	1		
Number of strains cultured	9	17	22
Number of infants with positive cultures	8	16	21

Two group A 3 non group A or B but not further serotyped

Prophylactic antibiotics were not used but the clinical indications for antibiotics were rather broad (Table 2) The physician in charge decided if the infants were to be treated in which case a combination of gentamicin and ampicillin was used except in two infants

Every second day during the time the catheter was in situ peripheral blood samples were obtained for anaerobic and aerobic cultures The samples were drawn by the vacutainer system directly into a supplemented peptone broth blood culture medium (Becton Dickinson Rutherford USA) At removal the distal part (1–2 cm) of the catheter was cut off and cultured in a 0 1% Dextrose broth At the same time an umbilical swab was cultured in the same type of broth The different bacteria were classified according to Cowan & Steel (11) Total white cells and platelets were analysed every second day Clinical signs and symptoms which could be referred to infection were specially noticed

RESULTS

The results of the bacteriological examinations are shown in Table 3 Only one infant had definite pathogenic bacteria in his blood culture

Table 4 *Culture results in relation to the degree of illness*

Degree of illness (score)	Blood culture			Catheter tip culture		
	No of infants	Positive culture		No of infants	Positive culture	
		n	%		*n*	%
1–2	37	5	13 5	38	11	28 9
3–4	19	3	15 8	18	5	27 8
5	5	0	0	2	0	0
Total	61	8	13 1	58	16	27 6

(*E coli*) but together with a presumably non pathogenic organism (*S epidermidis*) Five of the 8 infants with positive blood cultures later had a negative culture The cultures of the catheter tips showed presumably patho genic bacteria in all but one of the 16 positive cases

In 57 infants both blood and catheter cul tures were done None of them had the same bacteria in both cultures Cultures from the catheter tip as well as from the umbilicus were done in 52 infants Five of them had the same type of bacteria in both cultures but in 2 of the umbilical cultures there were 2 dif ferent strains

Cultures from the catheter tip were positive in two of 19 infants (11%) with and in 14 of 39 without antibiotic therapy (36%) Fourteen infants received antibiotic therapy before the last blood culture was taken One of them had a positive blood culture (7%) compared with 7 positive cultures among the 47 infants with out antibiotic therapy (15%) None of these differences were statistically significant how ever

The mean duration of the catheterization was almost the same 76 and 71 hours respec tively in infants with and without positive catheter tip culture Corresponding values for infants with and without positive blood culture were 92 and 65 hours respectively a non sig nificant difference however

Positive catheter tip cultures were found more often in side hole than in end hole cathe ters 32 and 20% respectively The difference was not statistically significant No difference was noticed between long and short catheters

The number of positive blood or catheter tip cultures was unrelated to the infants de gree of illness (Table 4)

Infants younger than 32 weeks of gestational age had an increased incidence of positive catheter tip cultures ($p<0\ 01$) but all their blood cultures were negative

Sixteen infants who developed arterial thromboses as described elsewhere (31) did not show an increased number of positive cul tures nor did the fourteen infants who re ceived blood transfusions through the cath eter

The infants who received breast milk late or not at all during the catheterization did not show an increased number of positive catheter or blood cultures

There was no difference with regard to the number of white cells or platelets between in fants with and without positive cultures

None of the infants had clinical signs or symptoms compatible with septicemia in com bination with a positive blood culture Nor had any of the infants who died any signs of bac terial infection at autopsy

DISCUSSION

Sepsis neonatorum is defined as a disease of infants who are less than 1 month of age are clinically ill and who have positive blood cul tures (20) The incidence is 1–3 cases per 1 000 live born infants (16 32)

Umbilical artery catheterization predisposes to infections (27 29) as does preterm delivery postnatal asphyxia and other adverse perinatal factors such as early rupture of the membranes complicated deliveries and resuscitation procedures (6, 7 18 21) All infants in the present study were high risk infants for neonatal septicemia Besides umbilical artery catheterization they had as a rule one or more predisposing factors

Previous prospective studies in which the frequency of infections in relation to umbilical artery catheterization has been studied are few Strauss et al (26) found sepsis in 2 of 26 infants (8%) and van Vliet et al (29) in 11 of 229 (5%) while others did not find any cases of sepsis at all (12 14) Krauss et al (22) who used arterial catheters of side hole type found positive catheter tip cultures in 6 of 11 infants (55%) while Powers et al (25) and Casalino et al (8) who used end hole catheters found positive catheter tip cultures in only 9 and 6% respectively Casalino et al suggested that these great differences could be due to the use of different types and positions of the catheter This explanation can be confirmed to some extent in the present study with a small but not significant difference in the frequency of positive catheter tip cultures between side and end hole catheters There was no such difference however with regard to the position of the catheter tip

The duration of the catheterization in relation to infection has previously been studied in connection with umbilical artery umbilical vein and peripheral vein catheters Thus several studies have shown a relation between the frequency of infection and the duration of the catheterization with umbilical vein catheters as well as with peripheral vein catheters (9 12) Several authors with one exception (27) have failed to demonstrate such a relation for umbilical artery catheters (4 22 29) The failure to show this relation in the present and earlier studies may depend on a relatively short mean duration of the catheterization period

The increased frequency of positive catheter tip cultures in the most preterm infants in the present investigation is in agreement with earlier findings (7 13 21)

An important question is the time for colonization of the catheters This might theoretically occur on insertion of the catheter during the time the catheter is in situ or on withdrawal of the catheter Previous studies have shown that the umbilicus is sterile in most cases during the first day of life but later a rapid colonization takes place (15 19) Once the umbilicus has been colonized umbilical cultures will as a rule remain positive in spite of intense cleaning (2) In the present study all infants had the umbilical artery catheter introduced during the first 12 hours but 9 infants were later recatheterized of whom 3 (33%) had a positive catheter tip culture Krauss et al (22) demonstrated bacterial growth inside the lumen of the catheters which speaks against colonization on withdrawal Colonization on insertion or withdrawal thus seems less likely so does the present finding that in only a few infants did the same bacteria grow from both the umbilicus and the catheter tip

The importance of a positive catheter tip culture is not clear The occurrence of bacterial growth on the catheter tip might enhance the risk for developing septicemia In spite of the high rate of positive catheter tip cultures the risk for septicemia seems to be small as shown by us and by others (4 12 22)

The importance of a positive blood culture from a newborn infant is also difficult to evaluate A positive blood culture might be an indication of contamination bacteremia or septicemia Eight infants (13%) had positive blood cultures to be compared with the findings of Albers et al (1) of 14% in healthy newborn infants They thought that positive cultures depend either on contamination or low grade self limited transient bacteremia This interpretation might hold also for our study since 7 of 8 positive blood cultures showed growth of bacteria that often are non pathogenic In the last culture two types of bacteria

grew out which might be an indication of a contamination

Several investigators have evaluated the effect of antibiotics as prophylaxis against infection in connection with umbilical artery catheterization In most of these studies as in the present antibiotics did not significantly decrease the frequency of positive blood or catheter tip cultures (5 12 22 29) Bard et al (4) however found in a randomized study a significantly increased number of positive blood and catheter tip cultures in non treated infants We agree with those authors including Bard et al (4) who believe that prophylactic antibiotics should not be used for umbilical artery catheterization (5 29) Prophylactic antibiotics can select antibiotic resistant strains (30) and delay a normal colonization of the intestines with increased risk for fungal infections (19)

The antibacterial effect of breast milk is well known (17) and the low incidence of severe infections in the present study might to some extent depend on the early feeding with human milk even if in the handling of the milk with freezing and heating some of the antimicrobial factors were destroyed (23)

The peripheral white blood cell count is a non specific test which may be difficult to interpret in newborn infants (7 18 32) Xantou (33) showed an increase in segmented and non segmented polymorphonuclear leucocytes in infected infants but she also found the total white cell count to be of limited value in the diagnosis However Tollner et al (28) found a significant increase in the total white cell count at the onset of septicemia and a marked drop when the disease advanced Thrombocytopenia is thought to be a relatively sensitive laboratory sign of septicemia in the neonatal period (10 18) In the present study there was no increase in leucocytes or decrease in thrombocytes in relation to positive cultures which speaks against the presence of septicemia in these infants

In conclusion we found that the risk of septicemia during umbilical artery catheterization is low if a strict aseptic technique is used in the catheterization and handling of the catheter The value of arterial catheterization in the control and the treatment of seriously ill newborn infants thus outweighs the possible risk of infection

ACKNOWLEDGEMENTS

All cultures were performed at the Department of Clinical Bacteriology University Hospital Linkoping This work was supported by a grant from Prenatalforskningsfonden

REFERENCES

1 Albers W H Tyler C W & Boxerbaum B Asymptomatic bacteremia in the newborn infant *J Pediatr 69* 193 1966
2 Anagnostakis D Kamba A Petrochilou V Arseni A & Matsaniotis N Risk of infection associated with umbilical vein catheterization *J Pediatr 86* 759 1975
3 Balagtas R C Bell C E Edwards L D & Levin S Risk of local and systemic infections associated with umbilical vein catheterization A prospective study in 86 newborn patients *Pediatrics 48* 359 1971
4 Bard H Albert G Teasdale F Doray B & Martineau B Prophylactic antibiotics in chronic umbilical artery catheterization in respiratory distress syndrome *Arch Dis Child 48* 630 1973
5 Bhatt D R Hodgman J E & Tatter D Evaluation of prophylactic antibiotics during umbilical catheterization in newborns *Clin Res 18* 217 1970
6 Blanc W A Pathways of fetal and early neonatal infection *J Pediatr 59* 473 1961
7 Buetow K C Klein S W & Lane R B Septicemia in premature infants *Am J Dis Child 110* 29 1965
8 Casalino M B & Lipsitz P J Contamination of umbilical catheters *J Pediatr 78* 1077 1971
9 Collins R N Braun P A Zinner S H & Kass E H Risk of local and systemic infection with polyethylene intravenous catheters A prospective study of 213 catheterizations *N Engl J Med 279* 340 1968
10 Corrigan J J Thrombocytopenia A laboratory sign of septicemia in infants and children *J Pediatr 85* 219 1974
11 Cowan S T & Steel K J *Manual for the identification of medical bacteria* New York 1974 2nd ed
12 Daschner F Adam D & Marget W Sepsis und Kontamination bei Nabel und Venenkathetern *Monatsch Kinderheilkd 122* 49 1974
13 Davies P A Bacterial infection in the fetus and newborn *Arch Dis Child 46* 1 1971
14 Dorand R D Cook L N & Andrews B F Umbilical vessel catheterization The low incidence of

Umbilical artery catheterization predisposes to infections (27 29) as does preterm delivery postnatal asphyxia and other adverse perinatal factors such as early rupture of the membranes complicated deliveries and resuscitation procedures (6 7 18 21) All infants in the present study were high risk infants for neonatal septicemia Besides umbilical artery catheterization they had as a rule one or more predisposing factors

Previous prospective studies in which the frequency of infections in relation to umbilical artery catheterization has been studied are few Strauss et al (26) found sepsis in 2 of 26 infants (8%) and van Vliet et al (29) in 11 of 229 (5%) while others did not find any cases of sepsis at all (12 14) Krauss et al (22) who used arterial catheters of side hole type found positive catheter tip cultures in 6 of 11 infants (55%) while Powers et al (25) and Casalino et al (8) who used end hole catheters found positive catheter tip cultures in only 9 and 6% respectively Casalino et al suggested that these great differences could be due to the use of different types and positions of the catheter This explanation can be confirmed to some extent in the present study with a small but not significant difference in the frequency of positive catheter tip cultures between side and end hole catheters There was no such difference however with regard to the position of the catheter tip

The duration of the catheterization in relation to infection has previously been studied in connection with umbilical artery umbilical vein and peripheral vein catheters Thus several studies have shown a relation between the frequency of infection and the duration of the catheterization with umbilical vein catheters as well as with peripheral vein catheters (9 12) Several authors with one exception (27) have failed to demonstrate such a relation for umbilical artery catheters (4 22 29) The failure to show this relation in the present and earlier studies may depend on a relatively short mean duration of the catheterization period

The increased frequency of positive catheter tip cultures in the most preterm infants in the present investigation is in agreement with earlier findings (7 13 21)

An important question is the time for colonization of the catheters This might theoretically occur on insertion of the catheter during the time the catheter is in situ or on withdrawal of the catheter Previous studies have shown that the umbilicus is sterile in most cases during the first day of life but later a rapid colonization takes place (15 19) Once the umbilicus has been colonized umbilical cultures will as a rule remain positive in spite of intense cleaning (2) In the present study all infants had the umbilical artery catheter introduced during the first 12 hours but 9 infants were later recatheterized of whom 3 (33%) had a positive catheter tip culture Krauss et al (22) demonstrated bacterial growth inside the lumen of the catheters which speaks against colonization on withdrawal Colonization on insertion or withdrawal thus seems less likely so does the present finding that in only a few infants did the same bacteria grow from both the umbilicus and the catheter tip

The importance of a positive catheter tip culture is not clear The occurrence of bacterial growth on the catheter tip might enhance the risk for developing septicemia In spite of the high rate of positive catheter tip cultures the risk for septicemia seems to be small as shown by us and by others (4 12 22)

The importance of a positive blood culture from a newborn infant is also difficult to evaluate A positive blood culture might be an indication of contamination bacteremia or septicemia Eight infants (13%) had positive blood cultures to be compared with the findings of Albers et al (1) of 14% in healthy newborn infants They thought that positive cultures depend either on contamination or low grade self limited transient bacteremia This interpretation might hold also for our study since 7 of 8 positive blood cultures showed growth of bacteria that often are non pathogenic In the last culture two types of bacteria

rew out which might be an indication of a contamination

Several investigators have evaluated the effect of antibiotics as prophylaxis against infection in connection with umbilical artery catheterization In most of these studies as in the present antibiotics did not significantly decrease the frequency of positive blood or catheter tip cultures (5 12 22 29) Bard et al (4) however found in a randomized study a significantly increased number of positive blood and catheter tip cultures in non treated infants We agree with those authors including Bard et al (4) who believe that prophylactic antibiotics should not be used for umbilical artery catheterization (5 29) Prophylactic antibiotics can select antibiotic resistant strains (30) and delay a normal colonization of the intestines with increased risk for fungal infections (19)

The antibacterial effect of breast milk is well known (17) and the low incidence of severe infections in the present study might to some extent depend on the early feeding with human milk even if in the handling of the milk with freezing and heating some of the antimicrobial factors were destroyed (23)

The peripheral white blood cell count is a non specific test which may be difficult to interpret in newborn infants (7 18 32) Xantou (33) showed an increase in segmented and non segmented polymorphonuclear leucocytes in infected infants but she also found the total white cell count to be of limited value in the diagnosis However Tollner et al (28) found a significant increase in the total white cell count at the onset of septicemia and a marked drop when the disease advanced Thrombocytopenia is thought to be a relatively sensitive laboratory sign of septicemia in the neonatal period (10 18) In the present study there was no increase in leucocytes or decrease in thrombocytes in relation to positive cultures which speaks against the presence of septicemia in these infants

In conclusion we found that the risk of septicemia during umbilical artery catheterization is low if a strict aseptic technique is used in the catheterization and handling of the catheter The value of arterial catheterization in the control and the treatment of seriously ill newborn infants thus outweighs the possible risk of infection

ACKNOWLEDGEMENTS

All cultures were performed at the Department of Clinical Bacteriology University Hospital Linkoping This work was supported by a grant from Prenatalforskningsfonden

REFERENCES

1 Albers W H Tyler C W & Boxerbaum B Asymptomatic bacteremia in the newborn infant *J Pediatr 69* 193 1966
2 Anagnostakis D Kamba A Petrochilou V Arseni A & Matsaniotis N Risk of infection associated with umbilical vein catheterization *J Pediatr 86* 759 1975
3 Balagtas R C Bell C E Edwards L D & Levin S Risk of local and systemic infections associated with umbilical vein catheterization A prospective study in 86 newborn patients *Pediatrics* 48 359 1971
4 Bard H Albert G Teasdale F Doray B & Martineau B Prophylactic antibiotics in chronic umbilical artery catheterization in respiratory distress syndrome *Arch Dis Child 48* 630 1973
5 Bhatt D R Hodgman J E & Tatter D Evaluation of prophylactic antibiotics during umbilical catheterization in newborns *Clin Res* 18 217 1970
6 Blanc W A Pathways of fetal and early neonatal infection *J Pediatr 59* 473 1961
7 Buetow K C Klein S W & Lane R B Septicemia in premature infants *Am J Dis Child 110* 29 1965
8 Casalino M B & Lipsitz P J Contamination of umbilical catheters *J Pediatr 78* 1077 1971
9 Collins R N Braun P A Zinner S H & Kass E H Risk of local and systemic infection with polyethylene intravenous catheters A prospective study of 213 catheterizations *N Engl J Med 279* 340 1968
10 Corrigan J J Thrombocytopenia A laboratory sign of septicemia in infants and children *J Pediatr 85* 219 1974
11 Cowan S T & Steel K J *Manual for the identification of medical bacteria* New York 1974 2nd ed
12 Daschner F Adam D & Marget W Sepsis und Kontamination bei Nabel und Venenkathetern *Monatsch Kinderheilkd 122* 49 1974
13 Davies P A Bacterial infection in the fetus and newborn *Arch Dis Child 46* 1 1971
14 Dorand R D Cook L N & Andrews B F Umbilical vessel catheterization The low incidence of

complications in a series of 200 newborn infants *Clin Pediatr 16* 569 1977
15 Fairchild J P Graber C D Vogel F H & Ingersoll R L Flora of the umbilical stump *J Pediatr 53* 538 1958
16 Gluck L Wood H F & Fousek M D Septicemia of the newborn *Pediatr Clin North Am 13* 1131 1966
17 Gothefors L Studies of antimicrobial factors in human milk and bacterial colonization of the newborn Umeå University Med diss 1975
18 Gotoff S P & Behrman R E Neonatal septicemia *J Pediatr 76* 142 1970
19 Habel A H Sandor G S Conn N K & McCrae W M Premature rupture of membranes and effects of prophylactic antibiotics *Arch Dis Child 47* 401 1972
20 Kaplan J M McCracken G H Bacterial and viral infections of the newborns *In* G B Avery (ed) *Neonatology Pathophysiology and Management of the Newborn* J P Lippincott Company Philadelphia 1975 p 542
21 Knudsen F U & Steinrud J Septicaemia of the newborn associated with ruptured foetal membranes discoloured amniotic fluid or maternal fever *Acta Paediatr Scand 65* 725 1976
22 Krauss A N Albert R F & Kannan M M Contamination of umbilical catheters in the newborn infant *J Pediatr 77* 965 1970
23 Leading article The special care of human milk *Br Med J II* 781 1978
24 Pildes R S Ramamurthy R S & Vidyasagar D Effect of triple dye on staphylococcal colonization in the newborn infant *J Pediatr 82* 987 1973
25 Powers W F & Tooley W H Contamination of umbilical vessel catheters Encouraging information *Pediatrics 49* 470 1972
26 Strauss A W Escobedo M & Goldring D Continuous monitoring of arterial oxygen tension in the newborn infant *J Pediatr 85* 254 1974
27 Symansky M R & Fox H A Umbilical vessel catheterization Indications management and evaluation of the technique *J Pediatr 80* 820 1972
28 Tollner U & Pohlandt F Septicemia in the newborn due to gram negative bacilli Risk factors clinical symptoms and hematologic changes *Europ J Pediatr 123* 243 1976
29 van Vliet P K J & Gupta J M Prophylactic antibiotics in umbilical artery catheterization in the newborn *Arch Dis Child 48* 296 1973
30 Weinstein L Superinfection A complication of antimicrobial therapy and prophylaxis *Am J Surg 10* 704 1964
31 Wesstrom G Finnstrom O & Stenport G Umbilical artery catheterization in newborns I Thrombosis in relation to catheter type and position *Acta Paediatr Scand 68* 575 1979
32 Wilson H D & Eichenwald H F Sepsis neonatorum *Pediatr Clin North Am 21* 571 1974
33 Xantou M Leucocyte blood picture in ill newborn babies *Arch Dis Child 47* 741 1972

Submitted Jan 25 1979
Accepted March 22 1979

(G W) Department of Paediatrics
University Hospital
S 581 85 Linkoping
Sweden

UMBILICAL ARTERY CATHETERIZATION IN NEWBORNS

III Thrombosis—A Study of Some Predisposing Factors

P HENRIKSSON G WESSTRÖM and ULLA HEDNER

From the Departments of Paediatrics University of Lund Allmanna Sjukhuset Malmo and University Hospital Linkoping and The Coagulation Laboratory Allmanna Sjukhuset Malmo Sweden

ABSTRACT Henriksson P Wesstrom G and Hedner U (Departments of Paediatrics University of Lund Allmanna Sjukhuset Malmo and University Hospital Linkoping and The Coagulation Laboratory Allmanna Sjukhuset Malmo Sweden) Umbilical artery catheterization in newborns III A study of some predisposing factors Acta Paediatr Scand 68 719 1979 —Thrombosis following umbilical artery catheterization is a relatively frequent complication Low fibrinolytic activity in the vessel walls high factor VIII and low antithrombin III (AT III Heparin cofactor activity) in blood are factors known to favour the formation of thrombosis In 30 newborns who died and in 2 foetuses the fibrinolytic activity determined in the aorta and the femoral vessels was in the normal adult range except for a few very immature infants and the foetuses The five cases with arterial thrombosis were not associated with low fibrinolytic activity The various factor VIII activities (VIII C VIIIR Ag and VIIIR RCF) and AT III were studied in 30 sick newborns and in 20 healthy newborns The sick exhibited increased levels of various factor VIII activities (VIIIR Ag and VIIIR RCF mainly) and markedly reduced levels of AT III The high factor VIII activities and the low AT III found will add to the existing risk of thrombosis due to the presence of a foreign material AT III substitution is suggested as a possible prophylactic

KEY WORDS Umbilical artery catheterization newborn thrombosis

Thromboembolic complications in connection with umbilical catheterization have received much space in the literature (6 11 22 25 36) Even spontaneous arterial and venous thrombosis occur in the newborn period the incidence varying between 1 and 5% (5 12 21 25 28)

It is generally accepted that low fibrinolytic activity in the vessel walls (19) and a low antithrombin III level (7 20) imply an increased risk of thrombosis Also an increase in the factor VIII protein is now thought to favour the development of thromboembolic disease (2 27 34)

Little is known about the variation of the above mentioned coagulant and fibrinolytic factors and their possible role in thrombosis in the newborn We therefore thought it worth while to determine the fibrinolytic activity in various vessels in foetuses and infants dying in the neonatal period and the factor VIII (VIII C VIIIR Ag VIIIR RCF) and antithrombin III (AT III) in healthy and sick newborns

CLINICAL MATERIAL

I *Sick newborns* (*n*=30) Four infants had IRDS (hyaline membrane disease) 9 unspecified respiratory symptoms 10 apnoea due to immaturity and 7 asphyxia Six infants developed thrombosis following catheterization of an umbilical artery a condition verified at angiography in 5 and autopsy in 1 Their gestational age ranged between 28 and 43 weeks (mean 34 3) The lowest birth weight was 1 000 and the highest 4 650 g (mean 2 087) A polyvinylchloride umbilical artery catheter was used in all 30 cases

II *Healthy newborns* (*n*=20) gestational ages 37-42 weeks (mean 39 6) Range of birth weights 2030-4760 g (mean 3370)

III *Neonatal autopsies* (*n*=30) Nine infants had IRDS (hyaline membrane disease) 6 apnoea due to immaturity 9 other pulmonary diseases 4 congenital malformations and 2 intra-uterine infections Eighteen infants had an indwelling umbilical artery catheter Five of them in

cluding one of the sick newborns had arterial thrombosis The gestational ages ranged from 24 to 42 weeks (mean 32 2) The lowest birth weight was 650 g and the highest 2 860 g (mean 2 130)

IV This group consisted of two *foetuses* obtained at legal abortion in the 17th and 20th week respectively Crown-heel lengths 19 and 23 cm

None of the newborns showed any bleeding symptoms

METHODS AND SAMPLING

Factor VIII clotting activity (VIII C) according to Nilsson et al (26) *factor VIII related antigen* (VIIIR Ag) according to Holmberg & Nilsson (17) *the ristocetin cofactor activity* (VIIIR RCF) as reported by Zuzel et al (38) and *antithrombin III* (AT III) with an immunochemical method described by Hedner & Nilsson (13)

Fibrinolytic activity in the vessel walls was determined in the way described by Pandolfi et al (30) The activity is expressed in arbitrary units Normal range in adult hand veins 6–10

In the sick newborns blood was obtained on day 1–4 via an indwelling umbilical artery catheter In the healthy newborns blood was taken from the external jugular vein on day 2–4 with the aid of a vacutainer system Informed consent had been given by the parents Sodium citrate 3 8% dilution 1 9 was used as anticoagulant and plasma was treated as previously described (26) Vessel specimens were obtained from the autopsied infants and the foetuses The specimens obtained post mortem were taken from the abdominal ($L_{3\ 5}$) and the thoracic part ($TH_{8\ 1}$) of aorta and from the femoral artery and vein They were frozen in fluid nitrogen and stored in −70°C until examined

RESULTS

The fibrinolytic activity in the aorta from the individual patients is shown in Fig 1 The foetuses and 8 of the 30 infants who died had low fibrinolytic activity in the aorta Six of them were born in the 24th to 32nd week Five infants were low in all vessels and one of them born in the 27th week had arterial thrombosis No difference in fibrinolytic activity was found

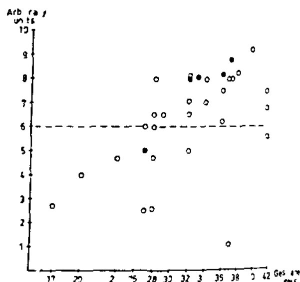


Fig 1 Fibrinolytic activity in the aorta (arbitrary units) in relation to the gestational age Filled circles denote cases with arterial thrombosis Dashed line denotes lower limit in adults

between the various vessels (Table 1) and no difference could be demonstrated between the proximal and the distal part of the aorta As is apparent from the figure the fibrinolytic activity did not vary with the presence or absence of thrombosis

The various activities of factor VIII are shown in Table 2 VIII C VIIIR Ag and VIIIR RCF in healthy infants significantly exceeded the adult levels ($p<0.001$ 0 001 and 0 01 respectively) VIII C did not differ significantly between the sick and healthy newborns VIIIR Ag and VIIIR RCF were however significantly higher in the sick ($p<0.05$) The various VIII activities did not seem to be influenced by the gestational age

AT III also shown in Table 2 was substantially lower in the sick newborns than in

Table 1 *Fibrinolytic activity in the vessel wall (arbitrary units mean and standard deviation)*

	n	Thor aorta	Abdom aorta	Femoral artery	Femoral vein
Neonatal autopsies	30	6 0 ±2 3	6 72 ±2 15	7 05 ±2 32	6 73 ±2 68
Foetuses	2	2 75 4 0	– –	– –	5 0 –

Table 2 *The various activities of factor VIII (VIII C VIIIR Ag VIIIR RCF) and antithrombin III (AT III) (mean and standard deviation)*

	VIII C (%)	VIIIR Ag (%)	VIIIR RCF (%)	AT III (%)
Healthy newborns	158 2 ±37 9	138 8 ±29 0	122 6 ±40 0	47 6 ±9 2
Sick newborns	169 9 ±87 3	174 1 ±56 5	147 5 ±37 7	34 3 ±17 6
Healthy adults	96 8 ±26 7	94 0 ±36 1	96 4 ±34 7	103 0 ±10 7

the healthy ($p<0\ 001$) who had only half the adult concentration Infants born before 34th week of gestation had significantly lower AT III levels than those born after that week ($p<0\ 05$)

The *factor VIII activities* and the *AT III* did not vary with the presence or absence of thrombosis

DISCUSSION

The development of thrombosis depends on several circumstances The presence of foreign material implies a risk for platelet adhesion to the foreign surface itself or to collagen exposed by injury of the vessel wall Furthermore a low fibrinolytic activity in the vessel wall and/or decreased ability to release such activity into the circulation in response to certain stimuli predispose to the development of thrombosis (19) Also conditions in the circulating blood including low inhibitors of coagulation and high levels of certain clotting factors may contribute to any predisposition to thrombotic disease Thus a decrease of AT III the inhibitor of thrombin is known to occur in families with severe thrombotic disease (1 7 20 24) The role of the factor VIII protein in primary haemostasis has recently been clarified It has been shown that factor VIII is important for the adhesion of platelets to the vessel wall (3 18 33) as well as for the formation of large platelet aggregates (39) It has also recently been found that of 148 patients all with recurrent deep venous thrombosis factor VIII protein (VIIIR Ag) was constantly increased in 29 (20%) (27)

The novel finding of a normal fibrinolytic activity in vessel walls in newborns was in accord with the earlier observation of a normal fibrinolytic activity in blood (8 10) The development of thrombotic complications did not seem to be related to the fibrinolytic activity in the vessel wall in these infants (Fig 1) The foetuses and the 6 very immature newborns had however a low fibrinolytic activity indicating a successive development an observation in agreement with what has been demonstrated for other components of the coagulation and fibrinolytic systems during foetal life (9 10 15 16)

Wesstrom et al (36) have recently shown that an umbilical artery catheter carries a significantly greater risk of causing thrombosis when its tip is placed low in the aorta (L_{3-5}) than when it is high (TH_{6-11}) Pandolfi et al (29) have reported a regional variation of the fibrinolytic activity in vessel walls namely a considerably higher activity in arm veins than in the leg veins a difference they used to explain why thrombosis occurs predominantly in the legs As we found no difference between various levels of the aorta the appearance of thrombosis mainly in the lower part of aorta could not be ascribed to any differences in fibrinolytic activity between different segments

As for AT III as expected (32) a decrease by about 50% was found immunochemically in healthy newborns compared with adults In

the sick newborns the AT III was decreased even more down to around 30% of the normal adult level This is in agreement with Weissbach et al (35) who used a biological method for determining AT III and with Mahasandana & Hathaway (23) The low AT III could be due to consumption during abnormal proteolysis and/or defective synthesis Abnormal proteolysis including both inactivation of the coagulation and the fibrinolytic systems has been demonstrated in sick newborns with the same basic diseases as those described in this paper (14)

The low AT III and the high factor VIII activities even more pronounced in sick newborns might help to explain the thrombotic complications often seen in connection with catheterization of the umbilical arteries On the other hand the finding of a normal fibrinolytic activity of the vessel walls offers protection against thrombosis and might partly explain the good prospects for those infants who develop thrombosis (4 31 37)

Heparin has been suggested as a prophylactic against thrombotic complications also in this age group The effect of systemic heparin in patients with low AT III (heparin cofactor activity) is limited Furthermore it has no inhibitory effect on the first step of the formation of the haemostatic plug and as known it increases the risk of bleeding complications Whether administration of purified AT III which is now commercially available (Kabi Stockholm Sweden) might prove to be the method of choice is a question that must abide future research

REFERENCES

1 Bjarke B Herin P & Blomback M Neonatal aortic thrombosis—a possible clinical manifestation of congenital antithrombin III deficiency *Acta Paediatr Scand 63* 297 1974
2 Bloom A L Giddings J C & Wilks C J Factor VIII on the vascular intima Possible importance in haemostasis *Nature* (*New Biol*) *241* 217 1973
3 Bloom A L & Peake I R Molecular genetics of factor VIII and its disorders *Semin Haematol 14* 319 1977
4 Boros S J Nystrom J F Thompson T R Reynolds J W & Williams H J Leg growth following umbilical artery catheterassociated thrombus formation A 4 year follow up *J Paediatr 87* 973 1975
5 Braly B D Neonatal arterial thrombosis and embolism *Surgery 58* 869 1965
6 Cochran W D Davis H T & Smith C Advantages and complications of umbilical artery catheterization in the newborn *Pediatrics 42* 69 1968
7 Egeberg O Inherited antithrombin deficiency causing thrombophilia *Thromb Diath Haemorrh 13* 516 1965
8 Ekelund H Hedner U & Nilsson I M Fibrinolysis in newborns *Acta Paediatr Scand 59* 33 1970
9 Ekelund H Hedner U & Åstedt B Fibrinolysis in human foetuses *Acta Paediatr Scand 59* 369 1970
10 Ekelund H & Finnstrom O Fibrinolysis in preterm infants and in infants small for gestational age *Acta Paediatr Scand 61* 185 1972
11 Goetzman B W Stadalnik R C Bogren H G Blankenship W J Ikeda R M & Thayer J Thrombotic complications of umbilical artery catheters A clinical and radiographic study *Pediatrics 56* 374 1975
12 Gross R E Arterial embolism and thrombosis in infancy *Am J Dis Child 70* 61 1945
13 Hedner U & Nilsson I M Antithrombin III in a clinical material *Thrombosis Research 3* 631 1973
14 Henriksson P & Ekelund H Abnormal proteolysis in sick newborns *Acta Paediatr Scand 64* 377 1975
15 Henriksson P Hedner U Nilsson I M Boehm J Robertson B & Lorand L Fibrin stabilizing factor (Factor XIII) in the fetus and the newborn infant *Pediatr Res 8* 789 1974
16 Holmberg L Henriksson P Ekelund H & Åstedt B Coagulation in the human fetus *J Pediatr 85* 860 1974
17 Holmberg L & Nilsson I M Immunologic studies in haemophilia A *Scand J Haematol 10* 12 1973
18 Hovig T & Stormorken H Ultrastructural studies on the platelet plug formation in bleeding time wounds from normal individuals and patients with von Willebrand s disease *Acta Pathol Microbiol Scand* Suppl 248 105 1974
19 Isacson S & Nilsson I M Defective fibrinolysis in blood and vein walls in recurrent idiopathic venous thrombosis *Acta Chir Scand 138* 313 1972
20 Johansson L Hedner U & Nilsson I M Familial antithrombin III deficiency as pathogenesis of deep venous thrombosis *Acta Med Scand 204* 491 1978
21 Knowlson G T & Marsden H B Aortic thrombosis in the newborn period *Arch Dis Child 53* 164 1978
22 Larroche J Cl Umbilical catheterization its complications *Biol Neonate 16* 101 1970
23 Mahasandana C & Hathaway W Circulating anticoagulants in the newborn relation to hypercoagulability and the idiopathic respiratory distress syndrome *Pediatr Res 7* 670 1973
24 Marciniak E Farley C H & de Simone P A

Familial thrombosis due to antithrombin III deficiency *Blood 43* 219 1974
25 Neal W A Reynolds J W Jarvis C W & Williams H J Umbilical artery catheterization Demonstration of arterial thrombosis by aortography *Pediatrics 50* 6 1972
26 Nilsson I M Blomback M & von Francken I On an inherited autosomal haemorrhagic diathesis with antihaemophilic globulin (AHG) deficiency and prolonged bleeding time *Acta Med Scand 159* 35 1957
27 Nilsson I M Hedner U & Nilsson B Factor VIII in thrombosis and intravascular coagulation Vth Congress on Thromboembolism Bologna 1978
28 Oppenheimer E H & Esterly J R Thrombosis in the newborn Comparison between infants of diabetic and nondiabetic mothers *J Pediatr 67* 549 1965
29 Pandolfi M Nilsson I M Robertson B & Isacson S Fibrinolytic activity of human veins *Lancet ii* 127 1967
30 Pandolfi M Isacson S & Nilsson I M Low fibrinolytic activity in the walls of veins in patients with thrombosis *Acta Med Scand 186* 1 1969
31 Powers W F & Swyer P R Limb blood flow following umbilical arterial catheterization *Pediatrics 55* 248 1975
32 Teger Nilsson A C Antithrombin in infancy and childhood *Acta Paediatr Scand 64* 624 1975
33 Tschopp T B Weiss H J & Baumgartner H R Decreased adhesion of platelets to subendothelium in von Willebrand s disease *J Lab Clin Med 83* 296 1974
34 Weiss H J Platelet physiology and abnormalities of platelet function *N Engl J Med 293* 580 1975
35 Weissbach G Domula M Lenk H & Schneider P The progressive antithrombin activity and its relations to other factors of the coagulation system in newborns *Acta Paediatr Scand 67* 555 1974
36 Wesstrom G Finnstrom O & Stenport G Umbilical artery catheterization in newborns I Thrombosis in relation to catheter type and position *Acta Paediatr Scand 68* 575 1979
37 Wesstrom G Umbilical artery catheterization in newborns V A follow up study To be published
38 Zuzel M Nilsson I M & Åberg M A method for measuring plasma ristocetin cofactor activity Normal distribution and stability during storage *Thrombosis Research 12* 745 1978
39 Åberg M & Rausing A The effect of Dextran 70 on the structure of ex vivo thrombi *Thrombosis Research 12* 1113 1978

Submitted Dec 27 1978
Accepted Febr 16 1979

(P H) Department of Paediatrics
Malmo Allmanna Sjukhus
S 21401 Malmo
Sweden



GASTRIC EMPTYING IN PRETERM INFANTS

BERTIL CAVELL

From the Department of Paediatrics University of Lund Lund Sweden

ABSTRACT Cavell B (Department of Paediatrics University of Lund Lund Sweden) Gastric emptying in preterm infants Acta Paediatr Scand 68 7?5 1979—The gastric emptying of meals of human milk and infant formula was studied in 11 healthy preterm (AGA) infants at a postnatal age of 1–9 weeks corresponding to 33–38 weeks of gestational age A total of 30 studies were performed using a marker dilution technique Gastric emptying of meals of human milk followed a biphasic emptying pattern with an initial fast phase In about 25 min half of the meal had left the stomach The emptying of meals of adapted infant formula usually followed a linear pattern and half the meal had left the stomach after 51 min After one hour an average of 24 8 ml of human milk and 19 2 ml of infant formula had left the stomach

KEY WORDS Gastric emptying preterm infants

In recent years there has been a growing interest in the feeding of the preterm infant Various techniques in providing these infants with an adequate nutritional supply e g nasogastric and transpyloric feeding have been suggested There are however reports on intestinal complications in preterm infants receiving transpyloric feedings (4 8 14) Nasogastric feeding is not associated with such complications This technique carries however the risk of regurgitation and aspiration of gastric contents when the volume of the meal exceeds the capacity of the infant s stomach for handling it On the other hand too small meal volumes may not fulfill the nutritional demands of the infant Knowledge of the reservoir function of the stomach of the preterm infant would thus seem important The purpose of the present study was to estimate quantitatively the gastric emptying function in healthy preterm infants fed complete meals

MATERIAL

Eleven preterm infants were studied at a postnatal age of one to nine weeks The clinical data on the infants are presented in Table 1 Gestational age was calculated from the first day of the mother s last menstrual period The degree of maturity was furthermore estimated by assessing external features neurological development and head circumference (5) Body surface was estimated according to Haycock et al (7) All infants were appropriate in weight for gestational age (AGA) according to Swedish standards (18) At the time of the studies all the infants were healthy and growing normally They were nursed in incubators and fed through a nasogastric infant feeding tube The studies were made after informed consent of the mothers

METHODS

General procedure Each infant was studied on one to five occasions making a total of 30 studies in the eleven infants The infants were given luke warm test meals of fresh frozen human milk usually obtained from their own mother or an adapted infant formula The composition of the infant formula used is shown in Table 2 The volume of the test meal was about 2? ml/kg body weight corresponding to the volume of the infant s ordinary meal

---

Part of this work was presented at The Joint Meeting of the European Society for Pediatric Gastroenterology and Nutrition & The North American Society for Pediatric Gastroenterology in Paris May 4–6 1978 (3)

Abbreviations IRDS = idiopathic respiratory distress syndrome RIS = respiratory insufficiency syndrome (15) TT = transient tachypnoea IPPV = intermittent positive pressure ventilation CPAP = continuous positive airway pressure ET = exchange transfusion

Table 1 *Clinical data in preterm infants*
For abbreviations see text

Patient no	Sex	Birth weight (g)	Gestational age at birth (weeks)	Previous history
1	f	950	29	Chylothorax IPPV 7 days CPAP 2 days
2	f	1 800	32	Twin TT
3	f	1 815	32	Twin
4	m	1 940	34	
5	f	960	29	RIS IPPV 9 days CPAP 2 weeks
6	f	910	28	IRDS IPPV 19 days CPAP 4 days
7	f	1 020	31	
8	m	900	28	IRDS RIS IPPV 6 weeks CPAP 3 hours
9	f	1 555	32	TT
10	f	1 280	32	Twin IRDS IPPV 1 day CPAP 4 days Hyperbilirubinemia ET
11	f	1 350	32	Twin IRDS CPAP 5 days

A French size 5 infant feeding tube was introduced into the stomach through the nose The gastric contents were withdrawn and the stomach was washed out with saline immediately before the test meal was injected During the study the infant was lying prone or in the right lateral position in an incubator

Amount of test meal in the stomach The amounts of test meal remaining in the stomach after various intervals were estimated using a modification of an earlier described procedure (2) A marker dilution technique is used after the infant has received a test meal containing polyethylene glycol (PEG Macrogol 3000) To an aliquot of the test meal is added a specified amount of PEG This aliquot is introduced into the stomach and mixed with the gastric contents by withdrawing and reinstilling with a syringe for 2 min Immediately before and after this procedure samples are withdrawn from the stomach for the estimation of PEG concentration

The first volume determination was thus performed 10–20 min after feeding had started The procedure was repeated once or twice at various intervals during one study

The volume of the gastric contents V is calculated from the equation

$$V = v_2 \frac{(C - c_2)}{(c_2 - c_1)} + v_1$$

where C = PEG concentration of test meal aliquot v_2 volume of test meal aliquot c_1 = PEG concentration of gastric contents before adding test meal aliquot c_2 = PEG concentration of gastric contents after adding test meal aliquot and v_1 = volume of sample of gastric contents withdrawn immediately before adding test meal aliquot The amount of test meal remaining in the stomach is estimated using the volume calculated according to the equation above and the changes of the PEG concentration during the study

After 50 to 100 min the procedure was finished by withdrawing the remaining gastric contents and washing out the stomach with 5 ml of saline The total residue of the meal was calculated using the PEG concentrations of the recovered gastric contents and of the wash out

The gastric quarter half and three-quarter-emptying times i e the time required to empty 25 50 and 75% of the test meal respectively were obtained by interpolation from individual emptying curves

Analytical method PEG was estimated turbidimetrically by the method of Hyden (10) using the modifications by Boulter & McMichael (1)

Test of validity The validity of the marker dilution technique in estimating known amounts of test meals of infant formula was tested *in vitro* A small amount of 0 1 molar hydrochloric acid was added to a vessel containing a known amount of infant formula and PEG After mixing the amount of infant formula in the vessel was estimated using the procedure described above A part of the contents of the vessel was removed another small amount of hydrochloric acid was added and the amount of infant formula remaining in the vessel was again estimated The

Table 2 *Data on infant formula*

Energy content	2 900 kJ/l
Osmolality	290 mOsm/kg
Composition	
Protein	16
Casein	6 5
Whey proteins	9 5
Fat	35
Lactose	72
Sodium	0 2
Potassium	0 7
Calcium	0 4
Phosphorus	0 26

Fat is derived from lard palm oil cottonseed oil and sunflower oil The amount of linoleic acid corresponds to 6% of the total energy content

Table 3 *Weighed versus experimentally estimated amounts of test meal in vitro*

Estimation no	Test meal (ml)		Difference (ml)
	Weighed	Estimated	
1	9 1	8 6	0 5
	19 7	17 1	2 6
3	11 4	11 9	−0 5
4	7 6	7 1	0 5
5	4 3	3 9	0 4

procedure was repeated three more times The amounts added to or removed from the vessel as well as the vessel including the test meal were weighed accurately The results of this test are presented in Table 3

Statistical methods Student s *t* test linear regression analysis and the Fisher exact probability test were used in the statistical analysis

RESULTS

Emptying patterns In 27 of the 30 studies gastric emptying had started at the first volume determination A biphasic emptying pattern with an initial rapid phase lasting some 20 min followed by a slower phase was found ı 19 of these 27 studies The emptying rate ppeared constant from the start in 5 studies ıcluding 4 performed with infant formula In studies (2 with human milk) only small mounts had emptied at the first determina ıon The subsequent emptying was faster

In two of the three remaining studies both with infant formula no emptying had occurred at the first determination 16 and 21 min after feeding respectively Following this initial delay gastric emptying proceeded with a constant rate in one study while in the other study the emptying curve was biphasic with an initial rapid phase

In the remaining study it was not possible to draw conclusions about the initial emptying as the first volume determination was invalidated by improper positioning of the feeding tube

In 16 studies where meals of human milk and infant formula were given to the same infant the biphasic emptying pattern with an initial fast phase was observed significantly more often than any of the other emptying patterns after meals of human milk than after meals of infant formula ($p = 0\ 024$)

The biphasic emptying pattern is illustrated in Fig 1 which shows the gastric emptying in 4 studies performed in one infant

Gastric emptying times The gastric emptying times for one quarter half and three quarters respectively of a test meal are presented individually in Table 4 The means and standard deviations of these emptying times are shown in Table 5

The quarter half and three quarter-emptying times for meals of infant formula were significantly longer than for meals of human milk ($p<0\ 001$)

No relationship was found between the time needed to administrate the test meal and the gastric quarter and half emptying times

Gastric emptying rate Sixty minutes after administration of the meal 24 8±7 5 ml of human milk meals and 19 2±4 7 ml of infant formula meals had left the stomach The difference is significant ($p<0\ 05$)

The corresponding figures calculated per 0 1 m^2 of body surface area are 19 4±4 5 ml and 13 8±2 8 ml respectively The difference is significant ($p<0\ 01$)

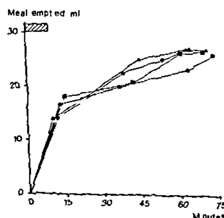


Fig 1 Gastric emptying in a 5 week-old preterm infant (patient no 6) after 30 ml test meals of human milk The amount of meal that has left the stomach is plotted against time Results are from four different studies performed on four consecutive days The shaded area indicates the mean duration of the administration of the meals

Table 4 *Gastric emptying times in 11 healthy preterm infants*
H = human milk F = infant formula lat = right lateral

Patient no	Age at time of study (weeks)	Body weight at time of study (g)	Body position	Test meal (ml)	Time for injecting test meal (min)	Time in min for emptying of specified fraction of test meal 25%	50%	75%
1	5	1 380	Lat	30 H	12	8	16	52
			Lat	30 H	12	8	15	73
			Lat	30 H	12	8	32	52
2	2	1 760	Lat	40 F	4	22	50	95
3	2	1 820	Lat	40 F	6	28	49	74
4	2	1 900	Lat	60 H	6	16	32	66
			Lat	50 H	4	12	23	33
5	8	1 600	Lat	30 H	8	7	30	50
	9	1 780	Lat	35 H	10	5	12	76
			Lat	35 H	15	24	36	79
6	4	1 180	Lat	30 H	9	7	15	36
	5	1 270	Prone	30 H	9	5	16	39
			Prone	30 H	12	7	13	49
			Prone	30 H	10	6	12	59
		1 420	Prone	30 F	11	36	54	79
7	2	1 075	Lat	25 H	7	11	36	54
			Lat	25 H	8		44	67
	3	1 180	Lat	30 H	10	6	12	27
8	6	1 390	Prone	25 H	7	14	27	40
	7	1 500	Prone	25 H	15	20	30	60
			Prone	25 F	5	28	48	66
9	1	1 450	Lat	25 H	7	18	34	64
10	2	1 410	Prone	25 H	12	8	16	64
			Prone	30 H	16	35	52	78
	3	1 510	Lat	30 F	13	34	58	76
	4	1 760	Prone	40 F	11	27	60	97
11	2	1 420	Prone	25 H	8	8	17	48
			Lat	30 H	15	12	32	72
	3	1 570	Prone	30 F	11	53	64	79
	4	1 760	Prone	40 F	9	12	32	74

Gastric emptying rate and postconceptional age of the infant were not significantly correlated ($r=0.31$, $p>0.05$). The rate of emptying was however related to the infant's body surface area ($r=0.43$, $p<0.02$) and body weight ($r=0.40$, $p<0.05$).

DISCUSSION

Comparison of methods Accurate data on gastric emptying of complete meals in the newborn and young infant are scarce. Earlier investigators have used radiological techniques in assessing gastric emptying of milk meals containing barium in infants (16). Because radiation hazards and poor correlation w more accurate methods (6) these techniqu are however unsuitable.

Gastric emptying has been studied in infa using the serial test meal (9). This techniq does not however allow one to follow t whole emptying course of a single meal. Th is possible using radioisotope techniques (1 The methods involved are however intricat

The marker dilution technique is simple a makes it possible to follow the gastric evacu tion of a single and complete meal during th whole postprandial phase. The validity of th

Table 5 *Gastric emptying of human milk and infant formula in preterm infants*
Mean values ±S D

Test meal	Gastric emptying time (min) Percent of test meal emptied 25	50	75
Human milk	11 7±7 5 (*n*=21)	25 1±11 5 (*n*=22)	51 3±16 7 (*n*=22)
Infant formula	30 0±11 9 (*n*=8)	51 9±9 8 (*n*=8)	80 0±10 7 (*n*=8)

marker dilution technique is demonstrated by the *in vitro* test where calculated amounts agree closely with known amounts

PEG has been used extensively as a volume indicator in studies of intestinal absorption and has also proved to be valid as a gastric non absorbable indicator for studies in man (11)

Gastric emptying pattern The biphasic emptying pattern which was found in most of the studies occurred significantly more fre quently after meals of human milk than after meals of infant formula which usually were associated with a linear emptying pattern or with an initial delay of gastric emptying The differences in emptying of the meals appear to e due to differences in the composition of uman milk and infant formula

Gastric emptying times Gastric emptying imes were short (Table 5) The differences in he emptying patterns between the two meals s reflected by the different gastric emptying imes which were significantly longer for neals of infant formula than for meals of human milk

The half time of the meal in the stomach observed in the present study is considerably shorter than the figure reported by Signer & Fridrich (17) in infants given a standard meal of infant formula This may at least in part be due to different meal volumes and different body positions being used in the two studies (19)

Gastric emptying rate The average amount of meal that had left the stomach during the first 60 min of a study was significantly larger for meals of human milk than for meals of infant formula Keller (12) in a study on gas tric emptying of human milk in infants found that the gastric emptying rate was constant and proportional to the infant s body surface area corresponding to an emptying of 22 ml per 0 1 m^2 body surface area per hour The emp tying rate for meals of human milk found in the present study agrees fairly well with this figure

Conclusion The present study shows that healthy preterm infants even if weighing slightly but above 1000 g have a rapid gas tric emptying after a feed of human milk Gas tric emptying times for meals of infant formula were roughly twice as long as those for meals of human milk

ACKNOWLEDGEMENT

This investigation was supported by grants from Semper Fund for Nutritional Research and The Swedish Baby Food Industry Fund for Nutritional Research The author wishes to thank Mrs Ann Mari Andersson for skilful techni al help The assistance of the staff of the Neonatal Unit Department of Paediatrics University Hospital Lund is gratefully acknowledged

REFERENCES

1 Boulter J M & McMichael H B Modification of poly-ethylene glycol estimation suitable for use with small animals *Gut 11* 268 1970
2 Cavell B Gastric emptying in infants *Lancet II* 904 1969
3 Cavell B Gastri emptying in low birth weight in fants *Acta Paediatr Belg 31* 176 1978
4 Chen J W & Wong P W K Intestinal complica tions of nasojejunal feeding in low birth weight in fants *J Pediatr 85* 109 1975
5 Finnstrom O Studies on maturity in newborn in fants VI Comparison between different methods for maturity estimation *Acta Paediatr Scand 61* 33 1972
6 Fridrich R Stalder G A Locher J & Heinis P Zur Erweiterung der Magendiagnostik mit nuklear medizinischen Verfahren *Dtsch Med Wochenschr 95* 2261 1970
7 Haycock G B Schwartz G J & Wisotsky D H Geometric method for measuring body surface area A height weight formula validated in infants children and adults *J Pediatr 93* 63 1978

Table 4 *Gastric emptying times in 11 healthy preterm infants*
H = human milk F = infant formula lat = right lateral

Patient no	Age at time of study (weeks)	Body weight at time of study (g)	Body position	Test meal (ml)	Time for injecting test meal (min)	Time in min for emptying of specified fraction of test meal		
						25%	50%	75%
1	5	1 380	Lat	30 H	12	8	16	52
			Lat	30 H	12	8	15	23
			Lat	30 H	12	8	32	52
2	2	1 780	Lat	40 F	4	22	50	95
3	2	1 820	Lat	40 F	6	28	49	74
4	2	1 900	Lat	60 H	6	16	32	66
			Lat	50 H	4	12	23	33
5	8	1 600	Lat	30 H	8	7	30	50
	9	1 780	Lat	35 H	10	5	12	26
			Lat	35 H	15	24	36	79
6	4	1 180	Lat	30 H	9	7	15	36
	5	1 270	Prone	30 H	9	5	16	39
			Prone	30 H	12	7	13	49
			Prone	30 H	10	6	12	59
		1 420	Prone	30 F	11	36	54	79
7	2	1 075	Lat	25 H	7	11	36	54
			Lat	25 H	8		44	62
	3	1 180	Lat	30 H	10	6	12	22
8	6	1 390	Prone	25 H	7	14	27	40
	7	1 500	Prone	25 H	15	20	30	60
			Prone	25 F	5	28	48	66
9	1	1 450	Lat	25 H	7	18	34	64
10	2	1 410	Prone	25 H	12	8	16	64
			Prone	30 H	16	35	52	78
	3	1 510	Lat	30 F	13	34	58	76
	4	1 760	Prone	40 F	11	27	60	97
11	2	1 420	Prone	25 H	8	8	17	48
			Lat	30 H	15	12	32	72
	3	1 570	Prone	30 F	11	53	64	79
	4	1 760	Prone	40 F	9	12	32	74

Gastric emptying rate and postconceptional age of the infant were not significantly correlated ($r=0.31$, $p>0.05$). The rate of emptying was, however, related to the infant's body surface area ($r=0.43$, $p<0.02$) and body weight ($r=0.40$, $p<0.05$).

DISCUSSION

Comparison of methods. Accurate data on gastric emptying of complete meals in the newborn and young infant are scarce. Earlier investigators have used radiological techniques in assessing gastric emptying of milk meals containing barium in infants (16). Because of radiation hazards and poor correlation with more accurate methods (6) these techniques are, however, unsuitable.

Gastric emptying has been studied in infants using the serial test meal (9). This technique does not, however, allow one to follow the whole emptying course of a single meal. This is possible using radioisotope techniques (17). The methods involved are, however, intricate.

The marker dilution technique is simple and makes it possible to follow the gastric evacuation of a single and complete meal during the whole postprandial phase. The validity of the

SMOKING DURING PREGNANCY—HEMATOLOGICAL OBSERVATIONS IN THE NEWBORN

A MEBERG P HÅGÅ H SANDE and O P FOSS

From the Department of Paediatrics the Department of Obstetrics and Gynaecology the Department of Clinical Chemistry and the Department of Pathology Ullevål Hospital Oslo Norway

ABSTRACT Meberg A Hågå P Sande H and Foss O P (Departments of Paediatrics Obstetrics and Gynaecology Clinical Chemistry and Pathology Ullevål Hospital Oslo Norway) Smoking during pregnancy—hematological observations in the newborn Acta Paediatr Scand 68 731 1979 —Hematological values were measured in 28 newborn infants of mothers smoking 10–20 cigarettes daily during pregnancy and in 25 infants of non-smokers Higher hematocrit levels were found on the 1st day of life in infants of smoking mothers (60 8±5 0% mean ±S D) compared to controls (57 5±4 8%) ($p<0\ 05$) The hematocrit levels correlated positively with the maternal smoking level (r–0.318 $p<0\ 05$) and the maternal serum thiocyanate concentrations at delivery (r=0.389 $p<0\ 01$) Cord serum values for erythropoietin serum iron transferrin and ferritin showed no statistically significant difference between the two groups A significant inverse correlation was found between the hematocrit value on the 1st day of life and the cord serum ferritin concentration ($r=-0\ 495$ $p<0\ 005$) The present results suggest that maternal smoking stimulates fetal erythropoiesis probably through a hypoxic effect on the fetus dose related to the maternal smoking level Increased erythropoiesis may cause increased iron incorporation into erythrocytes at expense of iron storage in the bone marrow and reticuloendothelial system

KEY WORDS Maternal smoking newborn infants hematocrit thiocyanate erythropoiesis ferritin

Maternal cigarette smoking during pregnancy may create a condition of chronic hypoxia for the fetus This may be due to impaired oxygen transport capacity because of carboxyhemoglobin production in mother and fetus (3) Carbon monoxide also causes a shift to the left of the oxyhemoglobin dissociation curve (3 16) impairing release of oxygen to the tissues Ultrastructural changes in the placenta (2) as well as decreased placental blood flow (17) may also impair oxygen supply to the fetus in smoking individuals Increased hemoglobin concentrations and elevated hematocrit levels were recently reported in infants of smoking mothers (4) In association with intrauterine growth retardation (IUGR) we have found transitory postnatal thrombocytopenia more frequently among infants of smoking mothers than of non smokers (13)

The purpose of the present investigation was to investigate hematocrit reticulocyte and thrombocyte counts erythropoietin (ESF) serum iron transferrin and ferritin levels in newborn infants of smoking and non smoking mothers and to relate these observations to the maternal smoking level

MATERIALS AND METHODS

Fifty three mothers (28 smoker and 25 non smokers) and their newborn infants were included in the series Consecutive smoking mothers were selected if they fulfilled the following criteria healthy women aged between 0 and 30 years in their first or second uncomplicated pregnancy a normal delivery at 39–42 weeks of gestation and a cigarette consumption estimated by the women themselves to be 10 15 or 20 cigarettes daily for the whole pregnancy The non smoking mothers selected were the first non smoking women admitted for delivery after a mother in the smoking group and that fulfilled all the other selection criteria Twenty five smokers and 23 non

8 Heird W C Nasojejunal feeding A commentary *J Pediatr 85* 111 1974
9 Husband J & Husband P Gastric emptying of water and glucose solutions in the newborn *Lancet II* 409 1969
10 Hydén S A turbidimetric method for the determination of higher polyethylene glycols in biological materials *Ann Roy Agric Coll Sweden 22* 139 1956
11 Ivey K J & Schedl H P Gastric nonabsorbable indicators for studies in man *Gastroenterology 59* 234 1970
12 Keller W Studies of the rapidity with which food leaves the stomach of infants *Acta Paediatr Scand 35* 180 1948
13 Landwirth J Continuous nasogastric infusion versus total intravenous alimentation *J Pediatr 81* 1037 1972
14 Pérez Rodrigues J Quero J Frias E G & Omenaca F Duodeno renal perforation in a neonate by a tube of silicone rubber during transpyloric feeding *J Pediatr 92* 113 1978
15 Raiha N C R Experience with neonatal intensive care in Finland *Ross Conference on Pediatric Research 59* 64 1968
16 Schnell N B Karelitz S & Epstein B S Radiographic study of gastric emptying in premature infants *J Pediatr 62* 342 1963
17 Signer E & Fridrich R Gastric emptying in newborns and young infants *Acta Paediatr Scand 64* 525 1975
18 Sterky G Swedish standard curves for intrauterine growth *Pediatrics 46* 7 1970
19 Yu V Y H Effect of body position on gastric emptying in the neonate *Arch Dis Child 50* 500 1975

Submitted Nov 30 1978
Accepted March 10 1979

Barnmedicinska kliniken
Lasarettet
S 221 85 Lund
Sweden

Acta Pædiatr Scand 68 731–734 1979

SMOKING DURING PREGNANCY—HEMATOLOGICAL OBSERVATIONS IN THE NEWBORN

A MEBERG P HÅGÅ H SANDE and O P FOSS

From the Department of Paediatrics the Department of Obstetrics and Gynaecology the Department of Clinical Chemistry and the Department of Pathology Ullevål Hospital Oslo Norway

ABSTRACT Meberg A Hågå P Sande H and Foss O P (Departments of Paediatrics Obstetrics and Gynaecology Clinical Chemistry and Pathology Ullevål Hospital Oslo Norway) Smoking during pregnancy—hematological observations in the newborn Acta Paediatr Scand 68 731 1979 —Hematological values were measured in 28 newborn infants of mothers smoking 10–20 cigarettes daily during pregnancy and in 25 infants of non smokers Higher hematocrit levels were found on the 1st day of life in infants of smoking mothers (60 8±5 0% mean ±S D) compared to controls (57 5±4 8%) ($p<0.05$) The hematocrit levels correlated positively with the maternal smoking level ($r=0.318$ $p<0.05$) and the maternal serum thiocyanate concentrations at delivery ($r=0.389$ $p<0.01$) Cord serum values for erythropoietin serum iron transferrin and ferritin showed no statistically significant difference between the two groups A significant inverse correlation was found between the hematocrit value on the 1st day of life and the cord serum ferritin concentration ($r=-0.495$ $p<0.005$) The present results suggest that maternal smoking stimulates fetal erythropoiesis probably through a hypoxic effect on the fetus dose related to the maternal smoking level Increased erythropoiesis may cause increased iron incorporation into erythrocytes at expense of iron storage in the bone marrow and reticuloendothelial system

KEY WORDS Maternal smoking newborn infants hematocrit thiocyanate erythropoiesis ferritin

Maternal cigarette smoking during pregnancy may create a condition of chronic hypoxia for the fetus This may be due to impaired oxygen transport capacity because of carboxyhemoglobin production in mother and fetus (3) Carbon monoxide also causes a shift to the left of the oxyhemoglobin dissociation curve (3 16) impairing release of oxygen to the tissues Ultrastructural changes in the placenta (2) as well as decreased placental blood flow (17) may also impair oxygen supply to the fetus in smoking individuals Increased hemoglobin concentrations and elevated hematocrit levels were recently reported in infants of smoking mothers (4) In association with intrauterine growth retardation (IUGR) we have found transitory postnatal thrombocytopenia more frequently among infants of smoking mothers than of non smokers (13)

The purpose of the present investigation was to investigate hematocrit reticulocyte and thrombocyte counts erythropoietin (ESF) serum iron transferrin and ferritin levels in newborn infants of smoking and non smoking mothers and to relate these observations to the maternal smoking level

MATERIALS AND METHODS

Fifty three mothers (28 smokers and 25 non smokers) and their newborn infants were included in the series Consecutive smoking mothers were selected if they fulfilled the following criteria healthy women aged between 20 and 30 years in their first or second uncomplicated pregnancy a normal delivery at 39–42 weeks of gestation and a cigarette consumption estimated by the women themselves to be 10 15 or 20 cigarettes daily for the whole pregnancy The non smoking mothers selected were the first non smoking women admitted for delivery after a mother in the smoking group and that fulfilled all the other selection criteria Twenty five smokers and 23 non

Table 1. *Hematocrit levels, reticulocyte and thrombocyte counts in capillary blood samples on the 1st and the 4th day of life in infants of smoking and non-smoking mothers*

Cigarette consumption ranged 10–20 cigarettes daily during pregnancy in the smoking group. *n* = number of observations

	Day after birth	Smokers			Non-smokers			*p*
		n	Range	Mean ± S.D.	*n*	Range	Mean ± S.D.	
Hematocrit (%)	1	25	49–75	60.8±5.0	23	48–67	57.5±4.8	<0.05
	4	22	52–68	59.9±5.2	20	47–66	56.4±5.6	>0.05 <0.10
Thrombocytes ($\times 10^9$/l)	1	25	187–405	290±54	23	162–363	285±47	>0.05
	4	23	160–395	286±67	20	152–360	273±52	>0.05
Reticulocytes (%)	1	25	1.3–3.1	2.2±0.41	22	1.5–2.9	2.2±0.34	>0.05
	4	23	0.5–1.9	1.0±0.3	18	0.5–1.3	0.9±0.3	>0.05
Decrease in reticulocyte number (%)	1–4	22	0.5–2.0	1.2±0.4	18	0.6–1.9	1.3±0.3	>0.05

smokers used iron supplements, and 25 smokers and 21 non-smokers used a multivitamin preparation during pregnancy according to a routine regimen. After delivery cords were clamped when pulsations in the umbilical cord arteries stopped.

Blood samples were taken from the neonates by heel punctures on the 1st and 4th day of life. The samples were analyzed for microhematocrit, reticulocyte (1 000 red cells counted after incubation with 1% brilliant cresyl blue) and thrombocyte counts (phase contrast microscopy). Cord blood samples were rapidly centrifuged and serum stored at −30°C until analyzed for serum iron, transferrin, ferritin and ESF. Serum iron was measured on a Technicon® autoanalyzer with the technique adjusted for 180 µl serum. Transferrin was measured by single radial immunodiffusion technique (M-Partigen®, Behring Institut, Germany) and ferritin by an immunoradiometric assay (Ria-Gnost Ferritin®, Behring Institut, Germany). ESF in cord serum was determined by a cell culture assay (5) measuring the number of erythroid colonies (CFU-E) formed in response to serum added to the cultures. The serum concentration used was 100 µl/ml and all determinations were done in duplicate. A step III preparation of sheep plasma ESF (Connaught Laboratories, Willowdale, Canada) was used as the standard.

Venous blood samples were taken from the mothers less than 24 hours before delivery. The samples were rapidly centrifuged and the serum stored at −30°C until analyzed for thiocyanate concentration by an automated modified method after Pettigrew & Fell (14). Informed consent from the mother was obtained in every case before blood samples were collected from them and their infants.

All blood samples were coded before analysis for every type of determination performed.

RESULTS

Table 1 shows the hematocrit, reticulocyte and thrombocyte counts in the newborn infants on the 1st and 4th day of life. Infants of smoking mothers had increased hematocrit levels on the 1st day of life compared to infants of non-smokers ($p<0.05$). The same differences were present on the 4th day of life with borderline significance ($0.10>p>0.05$). No differences were found in the reticulocyte and thrombocyte counts, and the fall in reticulocyte count from the 1st to the 4th day of life.

Maternal serum thiocyanate concentration at delivery (mean ± S.D.) was significantly elevated in smokers compared to non-smokers (109.6±29.9 µmol/l and 48.2±16 µmol/l, respectively, $p<0.01$), and the thiocyanate concentration correlated positively to the maternal smoking level ($r=0.80$, $p<0.001$). The infants' 1st day hematocrit values were found to correlate positively with the serum thiocyanate concentrations of the mothers (Fig. 1). A positive correlation was also found between the mothers' estimated cigarette consumption and their infants' 1st day hematocrit values ($r=0.318$, $p<0.05$).

No statistically significant differences were found between the infants of smoking and non-smoking mothers with regard to cord serum concentrations of serum iron (28.6±6.9 vs 29.2±5.5 µmol/l), transferrin (2.21±0.38 vs 2.22±0.30 g/l), ferritin (mean 276 vs 274 ng/ml, geometric mean ± S.D. 157–484 vs 120–

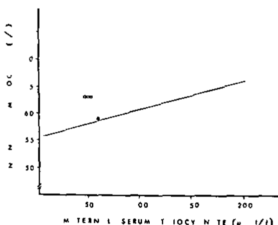


Fig 1 Relation between maternal serum thiocyanate concentrations at delivery and the hematocrit values of their infants on the 1st day of life in 3 smoking and 23 non smoking mothers

622 ng/ml) and ESF (980±351 vs 787±372 $CFU_e/2\times10$ cells)

A highly significant inverse correlation was found between the infants 1st day hematocrit values and their cord serum ferritin concentrations (Fig 2)

DISCUSSION

The increased hematocrit values found in infants of smoking mothers on the 1st day of life is in accordance with the findings of Garn & Shaw (4) This fits the hypothesis that maternal smoking creates a hypoxic condition for the fetus stimulating erythropoiesis

In adults a positive correlation is found between the hematocrit level and cigarette consumption (6) Thiocyanate is a detoxication product of cyanide absorbed from the smoke and is found to reflect the maternal smoking level (1 12) The hematocrit levels of neonates increased with increasing maternal serum thiocyanate concentration (Fig 1) and also with increasing cigarette consumption This indicates that the higher the maternal smoking level the stronger the hypoxic stimulus for fetal erythropoiesis

Hypoxia is a stimulus for ESF production During chronic hypoxia however serum ESF levels decrease towards pre hypoxic levels after an initial increase (15) Fetal growth may also be a potent stimulus for erythropoiesis (9) In infants with IUGR we have found that cord serum ESF levels were not different from the ESF levels in infants with birth weights appropriate for gestational age though IUGR infants had elevated hemoglobin concentrations (10) Chronic intrauterine hypoxia and a somewhat decreased growth rate of the fetus in smoking mothers therefore may be in accordance with normal cord serum ESF levels in these infants

Polycythemia is found in cigarette smokers with increase of the absolute red cell volume (16) In some individuals however a decreased plasma volume is found causing a relative polycythemia From the present data it cannot be excluded that a decreased plasma volume may contribute to the elevated hematocrit values found in newborn infants of smoking mothers

It has been suggested that infants of smoking mothers carry a larger iron reserve into the postnatal period than infants of non smokers (4) This may be correct as most of the infants iron reserves are contained in the red blood cells Iron stores in the bone marrow and the

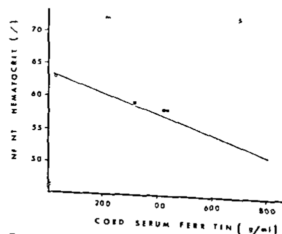


Fig 2 Relation between hematocrit values on the 1st day of life in 35 infants and their cord serum ferritin concentrations Seventeen of the infants had mothers smoking 10–20 cigarettes daily during pregnancy

reticuloendothelial system are reflected in the serum ferritin levels (8) As far as ferritin is concerned we found no difference in these iron stores in infants of smokers and non smokers This was also the case with regard to the serum iron and the transferrin concentrations in cord serum

The inversity found between the infants hematocrit values on the 1st day of life and *their cord serum ferritin concentrations (Fig* 2) suggests that increased red cell production may cause iron incorporation into erythrocytes at expence of iron storage in the bone marrow and the reticuloendothelial system

IUGR is often caused by a placental dysfunction syndrome with impaired oxygen supply to the fetus High hematocrit levels and a transitory postnatal thrombocytopenia frequently occur in these infants (13) The thrombocytopenia seems to be related to chronic intrauterine hypoxia (11) probably because of a competition on common stem cells for erythropoiesis and thrombopoiesis (7) In fetuses suffering from a placental dysfunction syndrome *maternal smoking may cause an additional* hypoxic stress This may explain why low thrombocyte counts more frequently occurred in neonates with IUGR of smoking mothers *than in IUGR infants of* non smokers (13) In normal pregnancy however the hypoxic stress on the fetus from maternal smoking may not exceed the compensatory mechanisms for thrombopoiesis

ACKNOWLEDGEMENTS

We would like to thank Grethe Berg Pedersen for excellent technical assistance This work is supported from The Norwegian Research Council for Science and the Humanities and from *The Norwegian Council on Cardiovascular* Diseases

REFERENCES

1 Andrews J Thiocyanate and smoking in pregnancy *Br J Obstet Gynaecol 80* 810 1973

2 Asmussen I *Ultrastructure of the* human placenta at term Observations on placentas from newborn children of smoking and non smoking mothers *Acta Obstet Gynecol Scand 56* 119 1977

3 Cole P V Hawkins L H & Roberts D Smoking during pregnancy and its effects on the fetus *Br J Obstet Gynaecol 79* 782 1972

4 Garn S M & Shaw H A Effect of maternal smoking on hemoglobins and hematocrits of the newborn *Am J Clin Nutr 31* 557 1978

5 Hågå P & Falkanger B In vitro assay for erythropoietin Erythroid colony formation in methylcellulose used for the measurement of erythropoietin in *plasma Blood in press*

6 Isager H & Hagerup L Relationship between cigarette smoking and high packed cell volume and hemoglobin levels *Scand J Haematol 8* 241 1971

7 Jackson C W & Edwards C C Biphasic thrombopoietic response to severe hypobaric hypoxia *Br J Haematol 35* 233 1977

8 Lanzkowski P Iron metabolism in the newborn infant *Clin Endocrinol Metabol 5* 149 1976

9 Matoth Y & Zaizov R Effect of intrauterine growth on erythropoiesis *Exp Hematol 6* 451 1978

10 Meberg A Unpublished data

11 Meberg A & Halvorsen S Transitory thrombocytopenia in small for-dates infants and in newborn mice exposed to hypobaric hypoxia cigarette smoke and CO gas inhalation during pregnancy *Proc Europ Soc Paediatr Res Annual Meeting* Turku 1978 p 58

12 *Meberg A Sande H Foss O P* & Stenwig J T Smoking during pregnancy—effects on the fetus and on thiocyanate levels in mother and baby *Acta Paediatr Scand in press*

13 Meberg A Örstavik I & Sovde A Erythroblastemia and thrombocytopenia in small for gestational age infants *Relation to intrauterine hypoxia infections* and maternal smoking *Acta Paediatr Belg 31* 213 1978

14 Pettigrew A R & Fell G S Simplified colorimetric determination of thiocyanate in biological fluids and *its application to investigation of the toxic amblyopias Clin Chem 18* 996 1972

15 Schooley J C & Mahlmann L J Hypoxia and the initiation of erythropoietin production *Blood Cells 1* 429 1975

16 Smith J R & Landaw S A Smokers polycythemia *N Engl J Med 298* 6 1978

17 Spira A Fournier R Grob J C Dreyfus J & Schwartz D Smoking during pregnancy and placental blood flow *Proc 6th Europ Congress of Perinatal Medicine* Vienna 1978

Submitted Jan 3 1979
Accepted March 29 1979

(A M) Department of Paediatrics
Ullevål Hospital
Oslo
Norway

Acta Pædiatr Scand 68 735–738 1979

INSULIN AND GLUCAGON SECRETION IN HEPATIC GLYCOGENOSES

SHINTARO OKADA YOSHIKI SEINO HIROKO KODAMA TORU YUTAKA KOJI INUI MAKOTO ISHIDA HYAKUJI YABUUCHI and YUTAKA SEINO

From the Department of Pediatrics Osaka University Hospital Osaka and the Third Division Department of Medicine University of Kobe School of Medicine Kobe Japan

ABSTRACT Okada S Seino Y Kodama H Yutaka T Inui K Ishida M Yabuuchi H and Seino Y (Department of Pediatrics Osaka University Hospital Fukushima ku Osaka and The Third Division Department of Medicine University of Kobe Kobe Japan) Insulin and glucagon secretion in hepatic glycogenoses Acta Paediatr Scand 68 735 1979 — Insulin and glucagon secretion was investigated in ten patients with hepatic glycogenosis types I and III in order to understand the relationship between hypoglycemia and pancreatic function In all patients both oral glucose tolerance and intravenous arginine infusion tests revealed hypoinsulinemia Decreased urinary C peptide levels with standard food intake also supported hypofunction of pancreatic beta cells On the contrary the normal secretion pattern of glucagon in both types indicated in the arginine loading test intact alpha cells in the pancreas Persistent hypoinsulinism which is apparently an adaptation to hypoglycemia could be an important cause of nutritional dwarfism in both types of glycogenosis The usefulness of the measurement of urinary C peptide which evaluates the pancreatic function and provides management for normal body growth is discussed

KEY WORDS Glycogenosis insulin C peptide glucagon

Hepatic glycogenoses are hereditary inborn errors of carbohydrate metabolism due to glycogenolytic enzyme deficiencies (6) Fasting hypoglycemia is frequently seen in patients with hepatic glycogenosis and it becomes a serious problem affecting the physical development of these patients Therefore it seems valuable to investigate the secretion of insulin and glucagon in this disorder because both hormones are important factors in the maintenance of blood glucose homeostasis

MATERIALS AND METHODS

Ten patients with hepatic glycogenosis seven males and three females aged 1 to 17 years were studied Among them seven cases were of type I (Glucose-6-phosphatase deficiency) and three cases were of type III (Amylo-1 6-glucosidase deficiency) The diagnosis was made on the basis of clinical manifestation and clinical history relevant enzyme assays in leukocytes and liver biopsied specimens (7) and glycogen content in erythrocytes (15)

After overnight fasting either glucose (? g/kg of body weight 40 g at maximum) was administered orally or a 10% solution of arginine (0 6 g/kg of body weight in 45 min) was administered intravenously In the former test heparinized blood was withdrawn at 0 30 60 90 and 120 min and in the latter at 0 5 15 30 45 60 and 90 min Each blood sample 2 ml was placed promptly into a chilled tube containing ?000 U of Trasylol in a volume of 0 2 ml and the plasma was separated as soon as possible with a refrigerated centrifuge The control group consisted of ten normal healthy children aged between 3 to 10 years All studies were carried out after obtaining informed parental consent

Plasma glucose was measured by the glucose oxidase method (5) plasma immunoreactive insulin (IRI) by the double antibody method of radioimmunoassay (4) and plasma pancreatic immunoreactive glucagon (IRG) by the talc method of radioimmunoassay with antiserum 30 K (14)

The urinary C peptide measurement was carried out by the method of Kuzuya et al (8) using freshly collected 24 hour urine samples Urine samples were usually assayed at 1 0 dilutions as Kuzuya et al (8) recommended Four patients with type I glycogenosis and three patients with type III 6–12 years of age were fed 70–80 cal/kg (10) which consisted of 50–60% carbohydrate 20–25% protein and 20–25% fat as Fernandes et al (?) suggested The renal function was intact in all cases of glycogenosis This finding excluded the influence of abnormal renal metabolism on the urinary excretion of C peptide Con

Supported in part by a grant from the Ministry of Education in Japan

reticuloendothelial system are reflected in the serum ferritin levels (8) As far as ferritin is concerned we found no difference in these iron stores in infants of smokers and non smokers This was also the case with regard to the serum iron and the transferrin concentrations in cord serum

The inversity found between the infants hematocrit values on the 1st day of life and their cord serum ferritin concentrations (Fig 2) suggests that increased red cell production may cause iron incorporation into erythrocytes at expence of iron storage in the bone marrow and the reticuloendothelial system

IUGR is often caused by a placental dysfunction syndrome with impaired oxygen supply to the fetus High hematocrit levels and a transitory postnatal thrombocytopenia frequently occur in these infants (13) The thrombocytopenia seems to be related to chronic intrauterine hypoxia (11) probably because of a competition on common stem cells for erythropoiesis and thrombopoiesis (7) In fetuses suffering from a placental dysfunction syndrome maternal smoking may cause an additional hypoxic stress This may explain why low thrombocyte counts more frequently occurred in neonates with IUGR of smoking mothers than in IUGR infants of non smokers (13) In normal pregnancy however the hypoxic stress on the fetus from maternal smoking may not exceed the compensatory mechanisms for thrombopoiesis

ACKNOWLEDGEMENTS

We would like to thank Grethe Berg Pedersen for excellent technical assistance This work is supported from The Norwegian Research Council for Science and the Humanities and from The Norwegian Council on Cardiovascular Diseases

REFERENCES

1 Andrews J Thiocyanate and smoking in pregnancy *Br J Obstet Gynaecol 80* 810 1973
2 Asmussen I Ultrastructure of the human placenta at term Observations on placentas from newborn children of smoking and non smoking mothers *Acta Obstet Gynecol Scand 56* 119 1977
3 Cole P V Hawkins L H & Roberts D Smoking during pregnancy and its effects on the fetus *Br J Obstet Gynaecol 79* 78? 1972
4 Garn S M & Shaw H A Effect of maternal smoking on hemoglobins and hematocrits of the newborn *Am J Clin Nutr 31* 557 1978
5 Hågå P & Falkanger B In vitro assay for erythropoietin Erythroid colony formation in methylcellulose used for the measurement of erythropoietin in plasma *Blood* in press
6 Isager H & Hagerup L Relationship between cigarette smoking and high packed cell volume and hemoglobin levels *Scand J Haematol 8* 241 1971
7 Jackson C W & Edwards C C Biphasic thrombopoietic response to severe hypobaric hypoxia *Br J Haematol 35* 233 1977
8 Lanzkowski P Iron metabolism in the newborn infant *Clin Endocrinol Metabol 5* 149 1976
9 Matoth Y & Zaizov R Effect of intrauterine growth on erythropoiesis *Exp Hematol 6* 451 19?8
10 Meberg A Unpublished data
11 Meberg A & Halvorsen S Transitory thrombocytopenia in small for-dates infants and in newborn mice exposed to hypobaric hypoxia cigarette smoke and CO gas inhalation during pregnancy *Proc Europ Soc Paediatr Res Annual Meeting* Turku 1978 p 58
12 Meberg A Sande H Foss O P & Stenwig J T *Smoking during pregnancy—effects* on the fetus and on thiocyanate levels in mother and baby *Acta Paediatr Scand* in press
13 Meberg A Örstavik I & Sovde A Erythroblastemia and thrombocytopenia in small for gestational age infants Relation to intrauterine hypoxia infections and maternal smoking *Acta Paediatr Belg 31* ?13 1978
14 Pettigrew A R & Fell G S Simplified colorimetric determination of thiocyanate in biological fluids and its application to investigation of the toxic amblyopias *Clin Chem 18* 996 1972
15 Schooley J C & Mahlmann L J Hypoxia and the initiation of erythropoietin production *Blood Cells 1* 429 1975
16 Smith J R & Landaw S A Smokers polycythemia *N Engl J Med 298* 6 1978
17 Spira A Fournier R Grob J C Dreyfus J & Schwartz D Smoking during pregnancy and placental blood flow *Proc 6th Europ Congress of Perinatal Medicine* Vienna 1978

Submitted Jan 3 1979
Accepted March 29 1979

(A M) Department of Paediatrics
Ullevål Hospital
Oslo
Norway

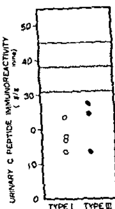


Fig. 3. Urinary C peptide levels are expressed as μg/g creatinine for type I (○) and type III (●) glycogenosis with shaded area as control level of mean ± standard deviation.

taking the recommended food with enough calories.

DISCUSSION

Though hormonal function seems important in glycogenoses in which hypoglycemia is one of the most significant symptoms, only a few reports were concerned with the study on the secretion of insulin and glucagon in the patients with hepatic glycogenosis. Lockwood et al. (9) reported abnormal glucose tolerance in five patients with type I glycogenosis and concluded that it was due to the insufficient secretion of insulin. Our results also indicated that the function of pancreatic beta cells in types I and III was below normal level in ordinary glucose tolerance test. Moreover we confirmed the insufficient function of beta cells with the direct stimulation with infused arginine. According to Lockwood et al. (9) during the adolescent period the decreased beta cell responsiveness might be an adaptive process and thus alleviate hypoglycemic attack. But because all cases with type I including three cases of early childhood clearly showed hypoinsulinemia, the adaptation seems to be completed promptly as early as the early infantile period and does not explain the clinical stability and improvement of hypoglycemia.

As Horwitz et al. (6) mentioned, assay of urinary C peptide is a useful method of integrated measurement of beta cell secretion for a long period. Approximately 5% of C peptide secreted by the beta cells appears in the urine as against only 0.1% of secreted insulin (6). Although the patients theoretically were given enough calories, urinary C peptide levels expressed as μg/g creatinine were significantly lower in both types than in the controls (Fig. 3). These data clearly indicated that the patients with hepatic glycogenosis suffered from chronic and mild deficiency of insulin.

The hypersecretion of glucagon was seen only in type I. In both types of hepatic glycogenosis the pancreatic alpha cells normally reacted to glucose and arginine loading tests. Hence hypoinsulinism may not directly cause the hypersensitivity of pancreatic alpha cells or hyperglucagonemia. Sadeghi Nejad et al. (13) reported the accelerated recycling process between glycogen and lactate in four patients of type I using a constant infusion of ^{14}C lactate. Thus, lactate which is present in increased amounts in the plasma with type I is interpreted as the source of glycogen synthesis for maintaining normoglycemic state. It favors hyperglucagonemia (1). The hormonal characterization in hepatic glycogenoses seems most likely to be limited to hyposensitivity of the pancreatic beta cells and independent of the function of alpha cells of the pancreas.

Insulin is a hormone which generally favors anabolic processes. It increases the availability of glucose for glycogenesis and lipogenesis and stimulates protein synthesis (16). Accordingly, if this abnormal hormonal situation, insulin deficiency, is prolonged for an extended period of time, it may cause the insufficiency of anabolic process and growth retardation in hepatic glycogenosis as in decompensated insulin-deficient diabetic children (11). Roe & Kogut (12) mentioned that the restoration of hypoglycemia caused normalization of the

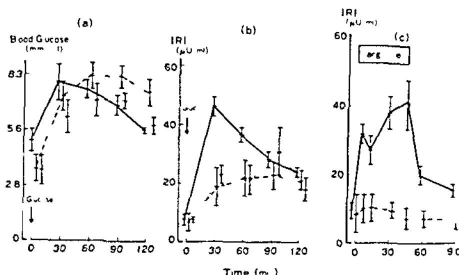


Fig 1 Response of blood glucose (*a*) and plasma immunoreactive insulin (IRI) (*b*) in the oral glucose tolerance test and response of plasma IRI in the arginine infusion test (*c*) Each bar represents the value of mean ± standard deviation for the control group (—) type I ( ) and type III glycogenosis ( ) In Fig 1*c* both types I and III are illustrated together (- -) since they did not show a different secretion pattern of IRI

trol values were obtained with urine samples from twenty age matched healthy children

RESULTS

In both types the fasting blood glucose was slightly lower than normal limits (3 7±0 9 mmol/l in type I 3 5±0 8 mmol/l in type III) and the maximum elevation of blood sugar was obtained between 60 and 90 min after glucose load The decrease of blood glucose after the peak was very slow as found in diabetes and was still 7 7±0 9 mmol/l at 120 min in type I (Fig 1*a*)

The basal value of IRI (9 5±3 3 μU/ml) was within normal limit But the maximum value of IRI after the oral glucose loading was less than 70% of that to the controls and its reaction was considerably delayed The highest value was obtained mostly at later than 60 min (Fig 1*b*) Here the straight relationship between blood glucose and IRI was shown

On the other hand before and after the arginine load the value of plasma IRG was generally high in type I although in type III it was within the control range (68±8 pg/ml) (Fig 2) Normal suppressive reaction of IRG after the oral glucose load was seen in all cases (data not shown)

Blood glucose was not changed with arginine loading in either type of hepatic glycogenosis IRI level of all patients was low and two phase secretion was not detected (Fig 1*c*) Quick response of IRG to arginine infusion was observed within 5 min in all cases (Fig 2)

Fig 3 shows urinary C peptide content in patients with glycogenosis and control subjects Patients with both types of glycogenosis excreted smaller amount of daily C peptide expressed as μg/g creatinine while they were

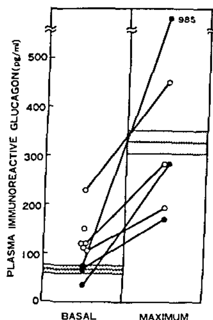


Fig 2 Plasma immunoreactive glucagon (IRG) levels at basal condition (left) and at maximum secretion after the arginine loading (right) are shown for type I glycogenosis (O) and type III glycogenosis (●) Shaded area is mean ± standard deviation of control value

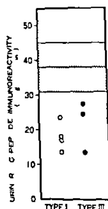


Fig 3 Urinary C peptide levels are expressed as μg/g creatinine for type I (O) and type III (●) glycogenosis with shaded area as control level of mean ± standard deviation

taking the recommended food with enough calories

DISCUSSION

Though hormonal function seems important in glycogenoses in which hypoglycemia is one of the most significant symptoms only a few reports were concerned with the study on the secretion of insulin and glucagon in the patients with hepatic glycogenosis Lockwood et al (9) reported abnormal glucose tolerance in five patients with type I glycogenosis and concluded that it was due to the insufficient secretion of insulin Our results also indicated that the function of pancreatic beta cells in types I and III was below normal level in ordinary glucose tolerance test Moreover we confirmed the insufficient function of beta cells with the direct stimulation with infused arginine According to Lockwood et al (9) during the adolescent period the decreased beta cell responsiveness might be an adaptive process and thus alleviate hypoglycemic attack But because all cases with type I including three cases of early childhood clearly showed hypoinsulinemia the adaptation seems to be completed promptly as early as the early infantile period and does not explain the clinical stability and improvement of hypoglycemia

As Horwitz et al (6) mentioned assay of urinary C peptide is a useful method of integrated measurement of beta cell secretion for a long period Approximately 5% of C peptide secreted by the beta cells appears in the urine as against only 0 1% of secreted insulin (6) Although the patients theoretically were given enough calories urinary C peptide levels expressed as μg/g creatinine were significantly lower in both types than in the controls (Fig 3) These data clearly indicated that the patients with hepatic glycogenosis suffered from chronic and mild deficiency of insulin

The hypersecretion of glucagon was seen only in type I In both types of hepatic glycogenosis the pancreatic alpha cells normally reacted to glucose and arginine loading tests Hence hypoinsulinism may not directly cause the hypersensitivity of pancreatic alpha cells or hyperglucagonemia Sadeghi Nejad et al (13) reported the accelerated recycling process between glycogen and lactate in four patients of type I using a constant infusion of ^{14}C lactate Thus lactate which is present in increased amounts in the plasma with type I is interpreted as the source of glycogen synthesis for maintaining normoglycemic state It favors hyperglucagonemia (1) The hormonal characterization in hepatic glycogenoses seems most likely to be limited to hyposensitivity of the pancreatic beta cells and independent of the function of alpha cells of the pancreas

Insulin is a hormone which generally favors anabolic processes It increases the availability of glucose for glycogenesis and lipogenesis and stimulates protein synthesis (16) Accordingly if this abnormal hormonal situation insulin deficiency is prolonged for an extended period of time it may cause the insufficiency of anabolic process and growth retardation in hepatic glycogenosis as in decompensated insulin deficient diabetic children (11) Roe & Kogut (12) mentioned that the restoration of hypoglycemia caused normalization of the

concentration of insulin and glucagon in serum But our results with oral glucose tolerance tests (Fig 1*a b*) indicated that the normalization of blood insulin level might need some longer preparative time because hyperglycemia after a single oral glucose loading brought only a slight increase of the serum insulin level Therefore the prolonged nocturnal intragastric infusion of glucose proposed by Greene et al (3) seems to be one of the best treatments for hepatic glycogenoses in order to restore the imbalance of hormones But the accurate clinical index to an adequate carbohydrate administration to the patients is not available at present The measurement of urinary C peptide may be a reliable index to maintain normal body growth of patients with hepatic glycogenosis

REFERENCES

1 Exton J H & Park C R Control of gluconeogenesis in liver II Effects of glucagon catecholamines and adenosine 3 5 monophosphate on gluconeogenesis in the perfused rat liver *J Biol Chem 243* 4189 1968

2 Fernandes J & Pikaar N A Hyperlipemia in children with glycogen storage disease *Am J Clin Nutr 22* 617 1969

3 Greene H L Slonim A E O Neill J A Jr & Burr I M Continuous nocturnal intragastric feeding for management of type I glycogen storage disease *N Engl J Med 294* 423 1976

4 Hales C N & Randle P J Immunoassay of insulin with antibody precipitate *Biochem J 88* 137 1963

5 Hill J B & Kessler G An automated determination of glucose utilizing a glucose oxidase peroxidase system *J Lab Clin Med 57* 970 1961

6 Horwitz D L Rubesstein A H Mako M E Cruz A & Blix P M C peptide in conditions other than diabetes mellitus *Diabetes ?7* ?67 1978

7 Howell R R The glycogen storage disease *In* J B Stanbury J B Wyngaarden & D S Fredrickson (eds) *The metabolic basis of inherited disease* McGraw Hill New York 1978 pp 137–159

8 Kuzuya T Matsuda A Sakamoto Y Tanabshi S & Kajinuma H C peptide immunoreactivity (CPR) in urine *Diabetes 27* 210 1978

9 Lockwood D H Merimee T J Edgar P J Greene M L Fujimoto W Y Seegmiller J E & Howell R R Insulin secretion in type I glycogen storage disease *Diabetes 18* 755 1969

10 National Nutritional Committee *Nutritional demand for Japanese and its explanation* Daiichi Shuppan Tokyo 1975 pp 23–48 (in Japanese)

11 Petersen H D Korgaard B Deckert T & Nielsen E Growth body weight and insulin requirement in diabetic children *Acta Paediatr Scand 67* 453 1978

12 Roe T F & Kogut M D The pathogenesis of hyperuricemia in glycogen storage disease type I *Pediatr Res 11* 664 1977

13 Sadeghi Nejad A Presente E Binkiewicz A & Senior B Studies in type I glycogenosis of the liver —the genesis and disposition of lactate *J Pediatr 85* 49 1974

14 Sakurai H & Imura H A sensitive radioimmunoassay for glucagon using talc method *Jap J Nucl Med 10* 135 1973

15 Sidbury J B Cornblath M Fisher J & House E Glycogen in erythrocytes of patients with glycogen storage disease *Pediatrics ?7* 103 1961

16 Tepperman J *Metabolic and endocrine physiology* Year Book Medical Publishers Chicago 1973 3rd ed pp 167–198

Submitted Oct 24 1978
Accepted Febr 19 1979

(S O) Department of Pediatrics
Osaka University Hospital
Fukushima ku
Osaka 553
Japan

SENSORY NERVE CONDUCTION VELOCITY AND VIBRATORY SENSIBILITY IN JUVENILE DIABETICS

Relationship to Endogenous Insulin

J LUDVIGSSON G JOHANNESSON L HEDING
A HAGER and Y LARSSON

From the Departments of Paediatrics and Neurophysiology University Hospital Linkoping Sweden and Novo Research Institute Bagsvaerd Denmark

ABSTRACT Ludvigsson J Johannesson G Heding L Hager A and Larsson Y (Departments of Paediatrics and Neurophysiology University Hospital Linkoping Sweden and Novo Research Institute Bagsvaerd Denmark) Sensory nerve conduction velocity and vibratory sensibility in juvenile diabetics Relationship to endogenous insulin Acta Paediatr Scand 68 739 1979 —Sensory nerve conduction velocity (NCV) and the vibratory sense (biothesiometry) were determined in 67 children and adolescents with insulin dependent diabetes Age at onset of diabetes varied between 1–14 years (mean ±S D 6 5±3 6) and the duration of diabetes between 4–17 years (7 7±3 4) Within ±3 months of the nerve function tests blood was drawn for determination of C peptide and insulin antibodies (IgG and IRI) A low NCV (<50 m/s) in the sural nerve and/or an abnormal vibratory sense (≥1 0 microns) were found in 34 patients (50 7%) Measurable fasting serum C peptide 0 04–0 60 pmol/ml (0 17±0 15) was found in 16 patients (23 9%) All but one patients had insulin antibodies with IgG 0 130–11 029 mU/ml (2 957±2 509) and total IRI 10–9120 µU/ml (1204±1723) In multiple regression analysis we did not find any correlation between nerve function and sex age or age at onset of diabetes and there was only a weak relationship between NCV and duration However there was a positive correlation between NCV and C peptide ($p<0\ 001$) Vibration sense was also better among patients with C peptide ($p<0\ 05$) The results support the view that insulin deficiency contributes to peripheral diabetic neuropathy

KEY WORDS Sensory nerve conduction velocity vibratory sense C peptide Insulin antibodies juvenile diabetes mellitus

Although neuropathy is said to be one of the most common of diabetic complications (8) little is known about its etiology Several hypotheses have been proposed and many clinical and experimental studies have been done on the subject However most such studies have been done on adults in which it may have been difficult to define onset of diabetes and where non diabetic factors interfere with the evaluation of purely diabetic factors such as insulin deficiency or occurrence of insulin antibodies

Clinical neuropathy is neither common nor pronounced in children with diabetes but neurophysiological studies of sensory function may reveal impairment of peripheral nerve function even in patients without neurological symptoms and signs (9 10 13 14)

The aim of this study was to study the occurrence of abnormal sensory nerve function among children and adolescents with diabetes and especially to analyze whether a normal function is associated with a preserved beta cell function

MATERIAL

The patients consisted of 67 children and adolescents aged 5–20 years 38 males and 29 females Age at onset of diabetes varied between 1–14 years (mean ±S D 6 4±3 6) and the duration of diabetes between 4–17 years (7 7±3 4) All patients were insulin dependent and were treated with insulin 1–2 times daily regular physical exercise and an optimal diabetic diet All patients except two tested their urine 2–4 times every day by the Clinitest method They noted the results in a diary which was shown to the doctor at the diabetic clinic 4–8 times a year Further characteristics of the material have been described in detail in previous publications (27)

Table 1 *Distribution of sensory nerve conduction velocity (NCV) amplitude of action potential and biothesiometry in 67 juvenile diabetics*

	N suralis		N medianus		N ulnaris	
	Left	Right	Left	Right	Left	Right
NCV						
<45 m/s	3	3	6	6	0	1
45–49	16	17	4	8	5	4
50–55	24	22	18	20	11	20
>55	17	19	37	30	49	41
Mean	52 4	52 2	55 7	54 3	58 5	57 4
S D	6 1	5 6	6 3	6 2	5 8	5 7
Range	34–69	37–63	41–69	39–71	46–72	44–69
Amplitude						
<10	38	39	7	9	19	24
10–20	18	18	35	30	38	35
>20	4	4	23	26	6	6
Mean	8 7	8 8	19 5	18 1	12 2	11 7
S D	5 6	5 2	9 3	8 9	5 1	5 1
Range	1–25	1 5–20 9	3 6–41 0	1 7–45	1 5–25	1 7–27 5
Biothesiometry						
<0 5 microns	15	14	19	15	17	17
0 5–0 99	26	31	30	38	32	38
≥1 0	14	13	8	6	9	5
Mean	0 80	0 76	0 66	0 62	0 60	0 67
S D	0 63	0 50	0 43	0 25	0 25	0 76
Range	0 25–3 70	0 25–2 90	0 30–3 2	0 20–1 05	0 2–1 2	0 30–1 40

METHODS

In the measurement of the sensory nerve conduction velocity (NCV) a digital signal averager (Biomac 1000) was used The stimulus duration used was 0 1 msec Spring loaded electrodes (15) were used for stimulation of the third finger (median nerve) and the fifth finger (ulnar nerve) The sural nerve was stimulated behind and below the lateral malleolus using circular chlorided silver electrodes The hands and the lower extremities were warmed when necessary using hot water bottles or sometimes immersion in warm water The skin temperature near the nerves was kept in the range 30–35 C in the hands and 30–40°C in the lower extremities

A supramaximal stimulation was used The sensory nerve action potentials were registered with conventional bipolar technique The signals were preamplified to the digital averager The averaged signals were written out on an X Y recorder whereafter the latency from the onset of the stimulus to the beginning of the first negative deflection could be measured manually as well as the peak to peak amplitude of the action potential

For the measurements of vibratory thresholds a biothesiometer (Ohio) was used The measurements were made on the tip of the third and fifth finger (median and ulnar nerves) and on the fifth toe (sural nerve) The biothesiometer is constructed so as to produce vibrations of a plastic tip with the double frequency of the domestic alternating current which in Sweden means 120 c/s (2× 60 c/s) The vibrator was placed against the finger tip or the toe with an even pressure The patient was told to report at the moment when he began to perceive the vibration while the voltage was slowly increased and to report the disappearance of the vibratory sensation as the voltage was decreased Several measurements were made in each case and mean values calculated from measurements for each individual finger or toe

On some occasion within ±3 months of the nerve function test blood was drawn from the fasting patients prior to their morning insulin injection Serum was stored at −18 C The binding of insulin to IgG was determined by the method of Christiansen (5) and total immunoreactive insulin (IRI) and serum C peptide according to Heding (19 20)

RESULTS

Clinical signs of neuropathy were found in 15 patients (22 4%) (mostly absence of deep tendon reflexes) on some occasion over a period of three years However only two of these patients experienced subjective symptoms In 12 of the 15 patients there was a low NCV (<50 m/s) in the sural nerve and/or abnormal vibratory sense measured by the biothesiometer (≥1 0 microns) Altogether 34 patients (50 7%) showed such abnormal neurophysiological values thus including 22 patients

Table 2 *Correlation between left and right side for each nerve regarding nerve conduction velocity amplitude biothesiometry*

	NCV		AMP		BIO	
	r	p	r	p	r	p
Suralis	+0 81	<0 001	0 71	<0 001	0 80	<0 001
Ulnaris	0 71	<0 001	0 66	<0 001	0 76	<0 001
Medianus	0 83	<0 001	0 65	<0 001	0 80	<0 001

without any clinical signs Table 1 shows the distribution of NCV amplitudes and biothesiometric values for the three nerves NCV and amplitude were lower and biothesiometry values higher for n suralis than for n medianus and n ulnaris There was a good correlation between the right and left sides of the respective nerves regarding both NCV amplitude and biothesiometry (Table 2) and the correlations for NCV between the different nerves were also rather high (sur/uln $r=0\ 66$ $p<0\ 001$ sur/med $r=0\ 45$ $p<0\ 001$ uln/med $r=0\ 65$ $p<0\ 001$) The correlation between NCV and amplitude was weakly positive at highest in medianus ($r=0\ 38$ $p<0\ 01$) and there was also a relationship between NCV and biothesiometric values in each single nerve (n suralis $r=0\ 34$ $p<0\ 02$) and between biothesiometry and amplitude (n suralis $r=0\ 46$ $p<0\ 001$) The correlation between NCV and skin temperature was positive (n suralis $r=0\ 45$ $p<0\ 001$)

A good or acceptable metabolic balance according to the number of urine tests without glucosuria in percentage of all tests performed (Table 3) was found in 53 patients (81 5%) out of those 65 who tested their urine regularly

Measurable C peptide was found in 16 patients (23 9%) between 0 04–0 60 pmol/ml (0 17±0 15) All patients except one had insulin antibodies with IgG 0 130–11 029 (2 957±2 509) mU/ml and total IRI 10–9 120 (1 204±1 723) μU/ml

In multiple regression analysis an effort was made to express NCV and biothesiometry as a function of several interdependent variables When skin temperature was held constant it was evident that sex and age at onset of diabetes played no role and even duration of diabetes was only weakly negatively correlated to NCV (n s) Neither did we find any significant relationship to glucosuria However there was a negative correlation between amount of insulin antibodies (IgG) and NCV ($p<0\ 01$) while the correlation between NCV and measurable fasting serum C peptide (≥0 04 pmol/ml) was significantly positive ($p<0\ 001$) Vibration sense evaluated by biothesiometry was not correlated to insulin antibodies but it was significantly better in those of lower age ($p<0\ 05$) and in patients with measurable C peptide ($p<0\ 05$)

DISCUSSION

From studies mainly on motor NCV several authors have reported peripheral neuropathy among diabetic children without any clinical symptoms or signs on routine neurological examination (6 9 13 14) We studied vibratory sense and sensory NCV as sensory nerve fibres seem to be affected before motor nerves (17 25) Our definition of low NCV as <50 m/s and abnormal vibratory sense as ≥1 0

Table 3 *Diabetic control*
Index = incidence of urine tests with less than 1% glucose

Index (%)	n	%
≥90	6	9 2
70–89	22	33 8
50–69	25	38 5
<50	12	18 5
$\bar{x}=67\ 6$	65	100 0

Table 1 *Distribution of sensory nerve conduction velocity (NCV) amplitude of action potential and biothesiometry in 67 juvenile diabetics*

	N suralis		N medianus		N ulnaris	
	Left	Right	Left	Right	Left	Right
NCV						
<45 m/s	3	3	6	6	0	1
45–49	16	17	4	8	5	4
50–55	24	22	18	20	11	20
>55	17	19	37	30	49	41
Mean	52 4	52 2	55 7	54 3	58 5	57 4
S D	6 1	5 6	6 3	6 2	5 8	5 7
Range	34–69	37–63	41–69	39–71	46–72	44–69
Amplitude						
<10	38	39	7	9	19	24
10–20	18	18	35	30	38	35
>20	4	4	23	26	6	6
Mean	8 7	8 8	19 5	18 1	12 2	11 7
S D	5 6	5 2	9 3	8 9	5 1	5 1
Range	1–25	1 5–20 9	3 6–41 0	1 7–45	1 5–25	1 7–22 5
Biothesiometry						
<0 5 microns	15	14	19	15	17	17
0 5–0 99	26	31	30	38	32	38
≥1 0	14	13	8	6	9	5
Mean	0 80	0 76	0 66	0 62	0 60	0 62
S D	0 63	0 50	0 43	0 25	0 25	0 26
Range	0 25–3 70	0 25–2 90	0 30–3 2	0 20–1 05	0 2–1 2	0 30–1 40

METHODS

In the measurement of the sensory nerve conduction velocity (NCV) a digital signal averager (Biomac 1000) was used The stimulus duration used was 0 1 msec Spring loaded electrodes (15) were used for stimulation of the third finger (median nerve) and the fifth finger (ulnar nerve) The sural nerve was stimulated behind and below the lateral malleolus using circular chlorided silver electrodes The hands and the lower extremities were warmed when necessary using hot water bottles or sometimes immersion in warm water The skin temperature near the nerves was kept in the range 30–35 C in the hands and 30–40°C in the lower extremities

A supramaximal stimulation was used The sensory nerve action potentials were registered with conventional bipolar technique The signals were preamplified to the digital averager The averaged signals were written out on an X Y recorder whereafter the latency from the onset of the stimulus to the beginning of the first negative deflection could be measured manually as well as the peak to peak amplitude of the action potential

For the measurements of vibratory thresholds a biothesiometer (Ohio) was used The measurements were made on the tip of the third and fifth finger (median and ulnar nerves) and on the fifth toe (sural nerve) The biothesiometer is constructed so as to produce vibrations of a plastic tip with the double frequency of the domestic alternating current which in Sweden means 120 c/s (2× 60 c/s) The vibrator was placed against the finger tip or the toe with an even pressure The patient was told to report at the moment when he began to perceive the vibration while the voltage was slowly increased and to report the disappearance of the vibratory sensation as the voltage was decreased Several measurements were made in each case and mean values calculated from measurements for each individual finger or toe

On some occasion within ±3 months of the nerve function test blood was drawn from the fasting patients prior to their morning insulin injection Serum was stored at −18°C The binding of insulin to IgG was determined by the method of Christiansen (5) and total immunoreactive insulin (IRI) and serum C peptide according to Heding (19 20)

RESULTS

Clinical signs of neuropathy were found in 15 patients (22 4%) (mostly absence of deep tendon reflexes) on some occasion over a period of three years However only two of these patients experienced subjective symptoms In 12 of the 15 patients there was a low NCV (<50 m/s) in the sural nerve and/or abnormal vibratory sense measured by the biothesiometer (≥1 0 microns) Altogether 34 patients (50 7%) showed such abnormal neurophysiological values thus including 22 patients

nomic nerve function in diabetic ketoacidosis *Lancet II* 167 1967
4 Chochinov R H Ullyot L E & Moorhouse J A Sensory perception thresholds in patients with juvenile diabetes and their close relatives *N Engl J Med 286* 1233 1972
5 Christiansen A H Radioimmunoelectrophoresis in the determination of insulin binding to IgG Methodological studies *Horm Metab Res 5* 147 1973
6 Eeg-Olofsson O & Petersén O Childhood diabetic neuropathy Clinical and neurophysiological study *Acta Paediatr Scand* 55 163 1966
7 Eliasson S G Nerve conduction changes in experimental diabetes *J Clin Invest 43* 2353 1964
8 Ellenberg M Diabetic neuropathy *In* J S Bajaj (ed) *Insulin and metabolism* Excerpta Medica Amsterdam 1977 p 109
9 Eng G D Hung N & August G P Nerve conduction velocity determination in juvenile diabetes *Diabetes in Juveniles Mod Probl Paediatr 12* 213 Karger Basel 1975
10 Fagerberg S E Diabetic neuropathy *Acta Med Scand 164* Suppl 345 1959
11 Fagerberg S E Petersen I & Wilhelmsen S G Motor disturbances in diabetes mellitus A clinical study using electromyography and nerve conduction velocity determination *Acta Med Scand 174* 711 1963
12 Gabbay K H Hyperglycemia polyol metabolism and complications of diabetes mellitus *Ann Rev Med* 26 521 1975
13 Gamstorp I Conduction velocity of peripheral motor nerves in mental retardation diabetes and various neurological diseases in childhood *Acta Paediatr Scand 53* 408 1964
14 Gamstorp I Shelburne S A Engleson G Redondo D & Traisman H S Peripheral neuropathy in juvenile diabetes *Diabetes 15* 411 1966
15 Goldberg M J Rogoff J B & Levine B Spring loaded electrode for sensory nerve conduction *Arch Phys Med Rehab 49* 665 1968
16 Gregersen G Diabetic neuropathy influence of age sex metabolic control and duration of diabetes on motor conduction velocity *Neurology (Minneap) 17* 972 1967
17 Gregersen G Vibratory perception thresholds and motor conduction velocity in diabetics and non-diabetics *Acta Med Scand 183* 61 1968
18 Green D A De Jesus P V Jr & Winegrad A I Effects of insulin and dietary myoinositol on impaired peripheral motor nerve conduction velocity in acute streptozotocin diabetes *J Clin Invest* 55 13 6 1975
19 Heding L G Determination of total serum insulin (IRI) in insulin treated diabetic patients *Diabetologia 8* 260 1972
20 Heding L G Radioimmunological determination of human C peptide in serum *Diabetologia 11* 541 1975
21 Kazibutowska A Polyneuropathy and motor fibre conduction velocity in children and adolescents with diabetes *Neur Neurochir Pol 8* 519 1974
22 Ludvigsson J *Metabolic control in juvenile diabetes mellitus* Linkoping University Medical Dissertation No 42 Zatatryckerierna Linkoping 1976
23 Ludvigsson J & Heding L G Beta-cell function in children with diabetes *Diabetes 27* Suppl 1 230 1978
24 Marcus J Ehrlich R Kelly M & Murphy E G Nerve conduction in childhood diabetes *C M A J 108* 1116 1973
25 La Montagne A & Buchtal F Electrophysiological studies in diabetic neuropathy *J Neurol Neurosurg Psychiatr 33* 442 1970
26 Schlaepfer W W Gerritsen G C & Dulin W E Segmental demyelination in the distal peripheral nerves of chronically diabetic Chinese hamsters *Diabetologia 10* 541 1974
27 Schmidt R E Matschinsky F M & Godfrey D A Fast and slow axoplasmic flow in sciatic nerve of diabetic rats *Diabetes 24* 1081 1975
28 Thomas P K & Eliasson S G (*In* P J Dyck P K Thomas & E H Lamberg (eds)) *Diabetic neuropathy peripheral neuropathy* WB Saunders Philadelphia 1975 vol 2 p 956
29 Ward J D Structure and biochemistry of degenerative lesions in diabetes mellitus Pathogenesis of diabetic neuropathy *Proceedings of the 8th Congress of the International Diabetes Federation* (ed W J Malaisse et al) Excerpta Medica Amsterdam 1974 p 419
30 Winegrad A I & Greene D A Diabetic polyneuropathy The importance of insulin deficiency hyperglycemia and alterations in myoinositol metabolism in its pathogenesis *N Engl J Med 295* 1416 1976

Submitted Febr 9 1979
Accepted March 10 1979

(J L) Department of Paediatrics
University Hospital
S 581 85 Linkoping
Sweden

microns of motion is chosen on the basis of the reports from other investigators (2 4) as well as from the results in non diabetics of our own laboratory We can conclude that many diabetic children without clinical symptoms and signs seemed to have abnormal sensory nerve function especially in the lower extremities This confirms previous clinical experience Already at the age of 7 years abnormal values were seen in some cases after a duration of only 4 years

The cause or causes of diabetic neuropathy are unknown Diabetic microangiopathy as a major cause (10) can probably be excluded as neurological disturbances are found very early after onset when no vascular complications other than possibly functional seem to exist (4) Most workers believe that the development of peripheral neuropathy is conditioned by some metabolic disturbance (8 28 29) There seems to be derangements both of the polyol pathway (12) and the myoinositol glucose and lipid metabolism (1) Eliasson (7) observed that in rats that were made hyperglycemic sensory NCV was impaired It has been shown that insulin treatment can prevent this impaired function only when it prevented prolonged periods of hyperglycemia (18 30) In rats acute diabetes causes decreased axonal transport These alterations can be reversed by insulin treatment (27) Furthermore it has been shown that in nerves with impaired motor NCV the concentration of free myoinositol is reduced which appears to be a consequence of insulin deficiency or hyperglycemia or both (30) Peripheral nerve lesions similar to those found in patients with overt diabetic polyneuropathy have been found in Chinese hamsters with spontaneous diabetes related to the duration and severity of hyperglycemia (26) Thus insulin deficiency and hyperglycemia seem to play primary roles in the pathogenesis of impaired nerve function in experimental animals with acute diabetes

The relevance of observations in experimental animals to juvenile diabetes in children cannot be assessed without clinical studies Other authors have reported a relation between disturbed nerve function and long duration of juvenile diabetes (9 13 14) and possibly poor metabolic control (6 14) Disturbed peripheral nerve function has been found during ketoacidosis (3) We have been able to measure the residual endogenous insulin secretion which better than conventional treatment protects the patients against insulin deficiency and thus contributes to less ketoacidosis and better metabolic balance (23) When taking several factors into consideration simultaneously in the multiple regression analysis we found that age was of no importance neither age at onset nor duration of diabetes in itself while abscence of C peptide which often but not always is seen after long duration and high amount of insulin antibodies were significantly negatively correlated to sensory NCV The correlation between residual insulin secretion and nerve function was also confirmed when biothesiometry was studied but insulin antibodies showed no such relationship As there is a negative correlation between C peptide and insulin antibodies (22) the negative relationship between insulin antibodies and NCV could be secondary to the association between C peptide and NCV Thus the role of insulin antibodies is uncertain However our results do support the view that a major cause of peripheral diabetic neuropathy is insulin deficiency

ACKNOWLEDGEMENTS

We want to thank Mr Bengt Berglov B A for his valuable help with the statistics The work was further supported by the Swedish Medical Research Council (proj nr 19X 4528) and by the Swedish Diabetic Association

REFERENCES

1 Anderson J W Metabolic abnormalities contributing to diabetic complications II Peripheral nerves *Am J Clin Nutr 29* 402 1976

2 Buchtal F & Rosenfalck A Evoked action potentials and conduction velocity in human sensory nerves *Brain Res 3* 1 1966

3 Campbell I W Ewing D J Harrower A B D Neilson J M M Fraser D M Baldwa V S Murray A & Clarke B F Peripheral and auto-

Acta Pædiatr Scand 68 745–749 1979

ON THE EPIDEMIOLOGY OF HUMAN TOXOPLASMOSIS IN SCANDINAVIA ESPECIALLY IN CHILDREN

GUNNEL HULDT R LAGERCRANTZ and P R SHEEHE

From the Swedish National Bacteriological Laboratory the Department of Paediatrics Karolinska Hospital Stockholm Sweden and the Department of Epidemiology Upstate University Syracuse N Y USA

ABSTRACT Huldt G Lagercrantz R and Sheehe P R (Swedish National Bacteriological Laboratory Department of Paediatrics Karolinska Hospital Stockholm Sweden and Department of Epidemiology Upstate University Syracuse N Y USA) On the epidemiology of human toxoplasmosis in Scandinavia especially in children Acta Paediatr Scand 68 745 1979 —A random sample representing approx one third of pre school children and approx 95% of school children in age groups 8 11 13 and 15 years in a suburb of Stockholm were examined for antibodies to Toxoplasma Antibodies were uncommon in small children They gradually appeared throughout childhood but especially in adolescence and adult life Antibodies were more common in females than in males Even small girls were more often infected than boys of corresponding age The difference becomes marked in puberty and is significant in adults A family study revealed evidence of familial aggregation of the infection albeit with borderline significance No correlation was found between occurrence of antibodies and the presence of cats in the families or reported consumption of raw meat The majority of representative samples of Lapps and Skolt Lapps in northern Scandinavia lacked antibodies to Toxoplasma

KEY WORDS Toxoplasma epidemiology family study

There is much about toxoplasmosis to excite all our fancies whether we be pediatricians internists ophthalmologists serologists parasitologists or shoe leather epidemiologists (Feldman 1960)

The toxoplasma parasite infects many animal species including man Infection is most often subclinical The infection rate in man varies considerably from country to country From 5 to 75% of the adult populations in different parts of the world are or have been infected (1–4 6–8 17)

The source of human infection was for a long time unknown However early observations indicated that toxoplasmosis may be transmitted by infected meat (12 18) and this was later confirmed by Desmonts et al (3) In 1969 Kean et al (13) could attribute a small epidemic of toxoplasmosis to the ingestion of hamburgers The role of the cat in the transmission of toxoplasmosis was first indicated by Hutchinson (10) who could demonstrate that cat faeces can be infective This observation led to detailed studies by several groups resulting in the present views that toxoplasma is a coccidia like parasite with the cat as final host and that the infective stage in the cat faeces is the oocyst (4 5 11 13 20)

Thus two routes of infection have been demonstrated It is however far from clear to what extent each of them contributes to human disease Nor do we know whether there may also be other ways by which man can be infected

We have studied the occurrence of toxoplasma antibodies in three populations 1 Children and adults in Sundbyberg a suburb of Stockholm Sweden 2 Skolt Lapps in northern Finland 3 Lapps in northern Norway We want to compare the incidence of the disease in these different populations and to elucidate the role of age sex domestic animals and family contact

Table 1 *Age and sex adjusted prevalences (%) of positive titers in families of positive and negative propositi by family size*

Family size	Persons (no)	Index Positive	Index Negative	Difference
1	13	46	46	0
	138	48	38	10
3	85	41	35	6
4	88	42	27	15
5	30	27	40	−13
Total	554	4	35	7

those of index negative ones as to age sex and family size The age and sex differences between families of the positive and negative propositi are very slight (Table 1) Of critical concern in this analysis are the distributions of the other family members

In families of sizes 2 3 or 4 which include the great majority of non index participants the age and sex adjusted prevalences were higher in the positive index children s families by from 6 to 15% The grand mean difference of 7% was obtained as the inverse variance weighted average of the differences for the 5 family size groupings The test of significance is based on the standard error or the weighted mean determined from *interfamilial* variability rather than individual variability

The 7% excess in the age sex and family size adjusted prevalence of positive individuals in families of positive index members was significant at the $p<0.06$ two-tailed level This is borderline since it does not quite reach the customary $p<0.05$ two tailed level of significance

The geometric mean titers (GMT) of those who were positive in the families of positive propositi was 51 while for those in families of negative propositi it was 45 Thus the ratio is 1 13 1 for the families of positive versus negative index children After adjustment for age and sex the ratio is 1 18 1 not significantly different from a ratio of 1 1 ($p<0.26$) (In the test of significance age and sex are taken into account and families rather than individuals are the analytical units)

While the excess prevalence of positivity in families of positive index children was of borderline significance ($p<0.06$) the excess GMT although not in itself significant ($p<0.26$) is supportive Thus in comparing the GMT's of all family participants excluding propositi there was a significant excess GMT in the families of positive propositi ($p<0.04$) This is evidence albeit with borderline significance of familial aggregation

Study of Lapps and Skolt Lapps

Antibody titers in different ethnic groups in northern Norway and Finland are presented in Table 2 In comparison with Sundbyberg there were very few infected individuals in the populations of Lapps and Skolt Lapps in the North

In contrast to the Sundbyberg families the Lappish populations live isolated This is true particularly for the Skolt Lapps who live in groups of two or three families within a large area Most of the houses are situated at a considerable distance from roads and the Lapps have to walk or ski to the nearest community The Lappish families live in close contact with their domestic animals By tradition all families have one or several dogs but also cats

Table 2 *Prevalence of toxoplasm antibodies in Lapps and Skolt Lapps according to sex and age*

Age	Females Total	Females DT pos	Males Total	Males DT pos
Lapps				
0–10	34	–	38	–
11–20	46	1	64	–
21–60	160	5	130	5
Sum	240	6 (5%)	232	5 (2 1%)
Skolt Lapps				
0–10	8	–	28	1
11– 0	40	–	60	4
21–60	57	6	63	2
Sum	125	6 (4 8%)	151	7 (4 6%)

MATERIAL AND METHODS

The study in Sundbyberg

Sundbyberg is a suburb of Stockholm Most inhabitants live in apartment houses with a good standard of living There are no slums All children are born in hospitals most infants and toddlers attend well baby clinics around 20% are in nursery schools School starts at 7 years of age and is compulsory for 9 years

A random sample representing approximately one third of families with pre school children and all families with school children in grades 2 (age 8–9 years) 5 (age 11–12 years) 7 (age 13–14 years) and 9 (age 15–16 years) were given a questionnaire concerning the health and development of the children and the occurrence of lymph adenopathy and eye symptoms The families were also asked if they had any domestic animal at home Five per cent of the families did not answer the questionnaire and did not participate in other parts of the study

Family study Parents to children who in the first study had dye test antibody titers of 250 or higher (positive index children) and parents to children with no demonstrable antibodies (negative index children) were contacted Altogether 554 individuals belonging to 205 families were examined Parents were interviewed (by G H and R L) regarding their own and their children s health signs of lymphadenopathy and eye diseases and habits of eating raw or inadequately prepared meat The children were examined by G H and R L

Study of Lapps and Skolt Lapps in northern Finland and Lapps in northern Norway

As part of an epidemiological study on tuberculosis 1 548 adult Lapps (≥15 years) in Kautokeino in northern Norway were screened Around a third of these individuals were examined for antibodies to echinococcus (9) and toxoplasma

In still another study 573 Lapps and 176 Skolt Lapps in Inari northern Finland were also examined for these antibodies

Serological tests Children were bled and sera stored at −70°C until tested in the toxoplasma dye test (DT) and complement fixation test at the National Bacteriological Laboratory Stockholm

RESULTS

Antibody and age The incidence of elevated antibody titers (DT≥10 and ≥250 respectively) in boys and girls of different ages in Sundbvberg are presented in Fig 1 Data are based on results in a third of the pre school population (probably representing a random sample) and around 95% of the school children Children below 1 year of age were not examined because their antibodies are most often passively acquired The adults are the parents to the index children in the family study

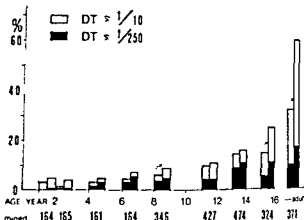


Fig 1 Frequency of positive dye test (DT) in children and adults in Sundbyberg related to age and sex

Antibodies to toxoplasma are uncommon in small children (Fig 1) They gradually appear especially in late childhood and adolescence In the 9th grade approximately 19% of the girls and 14% of the boys were infected Infection is however most often not acquired until adult life

Antibody and sex Antibodies were more common in females than in males (Fig 1) Even small girls were more often infected than boys of corresponding age The difference becomes marked in puberty and is significant in adults ($p<0\ 01$)

Antibodies and lymphadenopathy or eye symptoms Children with lymphadenopathy or eye symptoms did not differ serologically from the others

Antibodies and pets in the family Around 15% of the families had cats and 7% dogs 4% had birds (mostly canaries) and 5% other pets Families living with these animals did not differ in antibody titer from families without pets

Antibodies and the consumption of raw meat Around 10% of the families reported consumption of raw meat (mostly pork) by some or all family members These families did not differ in infection rate from others The quantities consumed are not known

Family study

We did not as planned succeed in matching families to index positive children against

4 No correlation between presence of antibodies and the exposure to cats or the eating of raw meat could be demonstrated

5 Toxoplasmosis is quite rare in Lapps and Skolt Lapps in northern Finland and Norway The reason for this could not be established

ACKNOWLEDGEMENT

Supported by a grant from Expressen s Foundation for Prenatal Research The competent and dedicated help of mrs Maria Svahn RN is gratefully acknowledged

REFERENCES

1 Beverley J K Fleck D G Kwantes W & Ludlam G B Age-sex distribution of various diseases with particular reference to toxoplasmic lymphadenopathy *J Hyg (Camb) 76* 215 1976

2 Christie E Dubey J P & Pappas P W Prevalence of Sarcocystis infection and other intestinal parasitisms in cats from a humane shelter in Ohio *J Am Vet Med Assoc 168* 421 1976

3 Desmonts G Couvreur J Alison F Baudelot J & Lelong M Etude epidemiologique sur la toxoplasmose de l'influence de la cuisson de viandes de boucherie sur la fréquence de l infection humaine *Rev Fr Etud Clin Biol 208* 1002 1969

4 Dubey J P Miller N L & Frenkel J K The Toxoplasma gondii oocyst from cat feces *J Exp Med 132* 636 1970

5 Dubey J P & Frenkel J K Cyst induced toxoplasmosis in cats *J P otozool 19* 155 1972

6 Feldman H A Epidemiological aspects of toxoplasmosis In *Human Toxoplasmosis* pp 169-174 Munksgaard Copenhagen 1960

7 Hamrbræus L Occurrence of antibodies against toxoplasma and tularense in a country of Northern Sweden *Communication 2nd Int Symp Circumpol Health Oulu* 1971 p 48

8 Hedenström G Huldt G & Lagercrantz R Toxoplasmosis in children *Acta Paediatr Scand 50* 304 1961

9 Huldt G Johansson S G O & Lantto S Echinococcosis in Northern Scandinavia Immune reactions in Kautokeino Lapps *Arch Environ Health 26* 36 1973

10 Hutchinson W M Experimental transmission of toxoplasma gondii *Nature (Lond) 206* 961 1965

11 Hutchinson W M Dunachie F Siim J C & Work K Coccidian like nature of Toxoplasma gondii *Br Med J i* 142 1970

12 Jacobs L Remington J S & Melton M L A survey of meat samples from swine cattle and sheep for the presence of encysted toxoplasma *J Parasitol 46* 23 1960

13 Kean B H Kimball A C L & Christenson W N An epidemic of acute toxoplasmosis *JAMA 208* 1002 1969

14 Korhonen P & Raisanen S Occurrence of toxoplasmosis in wild and domestic animals in Northern Finland *Communication 2nd Int Symp Circumpol Health Oulu* 1971 p 48

15 Kouba K Stehlíkova J Rajlichova J Ubner H & Svandova E Evaluation of some epidemiological factors in human toxoplasmosis *Z Gesamte Inn Med 31* 583 1976

16 Quilici M Ranque P Tounkara A & Rougemont A Toxoplasmosis in the republic of Mali *Acta Trop (Basel) 33* 239 1979

17 Siim J C A *Toxoplasmosis acquisita lymfadenosa* Munksgaard Copenhagen 1961

18 Weinman D & Chandler A H Toxoplasmosis in man and swine an investigation of the possible relationship *JAMA 161* 229 1956

19 Work K Toxoplasmosis With special reference to transmission and life cycle of Toxoplasma gondii *Acta Pathol Microbiol Scand (B) 221* 1 1971

20 Hutchison N Dunachie K Work K and Siim J C The life cycle of the coccidian parasite Toxoplasma gondii in the domestic cat *Trans R Soc Trop Med Hyg 65* 380 1971

Submitted Jan 20 1979
Accepted Febr 17 1979

(R L) Department of Paediatrics
Karolinska Sjukhuset
S 10401 Stockholm
Sweden

are common Among the Skolt Lapps practically each family has at least one cat

The Lapps also have different eating habits compared with the populations in other parts of Scandinavia Again this difference is most pronounced among the Skolt Lapps Fresh meat milk and eggs are rare and the main foods are fish bread and flour

DISCUSSION

Human toxoplasmosis is a fairly common disease in Sundbyberg but rare in Kautokeino and Inari in northern Scandinavia The reason for this is far from clear The populations studied vary with regard to ethnic origin and environmental factors It cannot be excluded that both climate conditions and isolation play a role for the low frequency of human infections in the North An interesting observation is the close contact with cats in the Lappish families In Stockholm around 50% of the cats are dye test positive (Huldt unpublished data) We have so far not been able to get permission from the Lapps to perform a serologic study on their cats However Korhonen et al (14) have shown that a wide variety of wild and domesticated animals in Finnish Lapland have antibodies to toxoplasma and it has been demonstrated that 60% of the reindeer in the north of Sweden were DT positive (Huldt unpublished) These findings indicate that toxoplasmosis might circulate in nature in the North but that owing to climate conditions or nutritional habits among the Lapps transmission from animal to man only seldom occurs

We could not demonstrate any correlation between the occurrence of toxoplasmosis and cats in the families in Sundbyberg Although our findings do not take into account the duration or intensity of exposure to cats it is difficult to see how the failure to estimate these quantitative factors could obscure a real relationship

Our results suggest a slight familial relationship of the disease It may be explained by genetic factors and/or a common source of infection within the homes If however inter human contact or common source of infection in the homes played a major role in the epidemiology we would expect a higher infection rate in the early childhood The relatively sharp rise in incidence in adult life suggests that the source of infection lies outside the homes (cf 15 16 19)

It has earlier been shown that in Scandinavian materials females are more often infected than males (8, 9) In our material the sex difference is observed already in small children and becomes marked in puberty and adolescence This may be due to hormonal differences and/or living habits However the fact that such sex distribution is not demonstrated in for instance materials from the United States speak against hormonal influence The theory that women are infected when eating raw meat could not be sustained in our study although this possibility cannot be excluded either It seems however that infected meat and oocysts from cat are not the only sources of infection

The disease is thus not uncommon in children and adolescents in Sundbyberg The infection was most often subclinical like in adults (6 17) An elevated antibody titer in a child should therefore not be interpreted as a sign of actual disease unless the child has clinical symptoms a relevant change in antibody titer combined with demonstration of specific Igm and/or IgA is a positive isolation of the parasite

SUMMARY

1 In Sundbyberg a suburb of Stockholm subclinical toxoplasmosis occurs already in early childhood and becomes more frequent in adolescence and especially in adult life
2 Girls and especially adult women are more often infected than boys and adult men
3 There is a familial clustering of the disease (albeit of doubtful statistical significance) but the data indicate that infection is most often acquired outside the home

4 No correlation between presence of antibodies and the exposure to cats or the eating of raw meat could be demonstrated

5 Toxoplasmosis is quite rare in Lapps and Skolt Lapps in northern Finland and Norway The reason for this could not be established

ACKNOWLEDGEMENT

Supported by a grant from Expressens Foundation for Prenatal Research The competent and dedicated help of mrs Maria Svahn RN is gratefully acknowledged

REFERENCES

1 Beverley J K Fleck D G Kwantes W & Ludlam G B Age-sex distribution of various diseases with particular reference to toxoplasmic lymphadenopathy *J Hyg (Camb)* 76 215 1976

2 Christie E Dubey J P & Pappas P W Prevalence of Sarcocystis infection and other intestinal parasitisms in cats from a humane shelter in Ohio *J Am Vet Med Assoc 168* 421 1976

3 Desmonts G Couvreur J Alison F Baudelot J & Lelong M Etude épidémiologique sur la toxoplasmose de l'influence de la cuisson de viandes de boucherie sur la fréquence de l'infection humaine *Rev Fr Etud Clin Biol 208* 100_ 1969

4 Dubey J P Miller N L & Frenkel J K The Toxoplasma gondii oocyst from cat feces *J Exp Med 13* 636 1970

5 Dubey J P & Frenkel J K Cyst induced toxoplasmosis in cats *J Proto ool 19* 155 1972

6 Feldman H A Epidemiological aspects of toxoplasmosis In *Human Toxoplasmosis* pp 169–174 Munksgaard Copenhagen 1960

7 Hambræus L Occurrence of antibodies against toxoplasma and tularense in a country of Northern Sweden *Communication 2nd Int Symp Circumpol Health Oulu* 1971 p 48

8 Hedenström G Huldt G & Lagercrantz R Toxoplasmosis in children *Acta Paediatr Scand 50* 304 1961

9 Huldt G Johansson S G O & Lantto S Echinococcosis in Northern Scandinavia Immune reactions in Kautokeino Lapps *Arch Environ Health 26* 36 1973

10 Hutchinson W M Experimental transmission of toxoplasma gondii *Nature (Lond) 206* 961 1965

11 Hutchinson W M Dunachie F Siim J C & Work K Coccidian like nature of Toxoplasma gondii *Br Med J i 14* 1970

12 Jacobs L Remington J S & Melton M L A survey of meat samples from swine cattle and sheep for the presence of encysted toxoplasma *J Parasitol 46* 23 1960

13 Kean B H Kimball A C L & Christenson W N An epidemic of acute toxoplasmosis *JAMA 208* 1002 1969

14 Korhonen P & Raisanen S Occurrence of toxoplasmosis in wild and domestic animals in Northern Finland *Communication 2nd Int Symp Circumpol Health Oulu* 1971 p 48

15 Kouba K Stehlíkova J Rajlichova J Ubner H & Svandova E Evaluation of some epidemiological factors in human toxoplasmosis *Z Gesamte Inn Med 31* 583 1976

16 Quilici M Ranque P Tounkara A & Rougemont A Toxoplasmosis in the republic of Mali *Acta Trop (Basel) 33* 239 1979

17 Siim J C A *Toxoplasmosis acquisita lymfadenosa* Munksgaard Copenhagen 1961

18 Weinman D & Chandler A H Toxoplasmosis in man and swine an investigation of the possible relationship *JAMA 161* 229 1956

19 Work K *Toxoplasmosis* With special reference to transmission and life cycle of Toxoplasma gondii *Acta Pathol Microbiol Scand (B)* 221 1 1971

20 Hutchison N Dunachie K Work K and Siim J C The life cycle of the coccidian parasite Toxoplasma gondii in the domestic cat *Trans R Soc Trop Med Hyg* 65 380 1971

Submitted Jan 20 1979
Accepted Febr 17 1979

(R L) Department of Paediatrics
Karolinska Sjukhuset
S 10401 Stockholm
Sweden

are common Among the Skolt Lapps practically each family has at least one cat

The Lapps also have different eating habits compared with the populations in other parts of Scandinavia Again this difference is most pronounced among the Skolt Lapps Fresh meat milk and eggs are rare and the main foods are fish bread and flour

DISCUSSION

Human toxoplasmosis is a fairly common disease in Sundbyberg but rare in Kautokeino and Inari in northern Scandinavia The reason for this is far from clear The populations studied vary with regard to ethnic origin and environmental factors It cannot be excluded that both climate conditions and isolation play a role for the low frequency of human infections in the North An interesting observation is the close contact with cats in the Lappish families In Stockholm around 50% of the cats are dye test positive (Huldt unpublished data) We have so far not been able to get permission from the Lapps to perform a serologic study on their cats However Korhonen et al (14) have shown that a wide variety of wild and domesticated animals in Finnish Lapland have antibodies to toxoplasma and it has been demonstrated that 60% of the reindeer in the north of Sweden were DT positive (Huldt unpublished) These findings indicate that toxoplasmosis might circulate in nature in the North but that owing to climate conditions or nutritional habits among the Lapps transmission from animal to man only seldom occurs

We could not demonstrate any correlation between the occurrence of toxoplasmosis and cats in the families in Sundbyberg Although our findings do not take into account the duration or intensity of exposure to cats it is difficult to see how the failure to estimate these quantitative factors could obscure a real relationship

Our results suggest a slight familial relationship of the disease It may be explained by genetic factors and/or a common source of infection within the homes If however interhuman contact or common source of infection in the homes played a major role in the epidemiology we would expect a higher infection rate in the early childhood The relatively sharp rise in incidence in adult life suggests that the source of infection lies outside the homes (cf 15, 16 19)

It has earlier been shown that in Scandinavian materials females are more often infected than males (8 9) In our material the sex difference is observed already in small children and becomes marked in puberty and adolescence This may be due to hormonal differences and/or living habits However the fact that such sex distribution is not demonstrated in for instance materials from the United States speak against hormonal influence The theory that women are infected when eating raw meat could not be sustained in our study although this possibility cannot be excluded either It seems however that infected meat and oocysts from cat are not the only sources of infection

The disease is thus not uncommon in children and adolescents in Sundbyberg The infection was most often subclinical like in adults (6 17) An elevated antibody titer in a child should therefore not be interpreted as a sign of actual disease unless the child has clinical symptoms a relevant change in antibody titer combined with demonstration of specific Igm and/or IgA is a positive isolation of the parasite

SUMMARY

1 In Sundbyberg a suburb of Stockholm subclinical toxoplasmosis occurs already in early childhood and becomes more frequent in adolescence and especially in adult life
2 Girls and especially adult women are more often infected than boys and adult men
3 There is a familial clustering of the disease (albeit of doubtful statistical significance) but the data indicate that infection is most often acquired outside the home

Acta Pædiatr Scand 68 751–758 1979

A BOY WITH SEVERE INFANTILE GASTROGEN LACTOSE INTOLERANCE AND ACQUIRED LACTASE DEFICIENCY

N O BERG A DAHLQVIST and T LINDBERG

From the Departments of Pathology and Nutrition University of Lund and Department of Paediatrics Malmo General Hospital Malmo University of Lund Sweden

ABSTRACT Berg N O Dahlqvist A and Lindberg T (Departments of Pathology and Nutrition University of Lund and Department of Paediatrics Malmo General Hospital Malmo University of Lund Sweden) A boy with severe infantile gastrogen lactose intolerance and acquired lactase deficiency Acta Paediatr Scand 68 751 1979 —A 10 year-old boy with severe familial lactose intolerance in infancy (vomiting failure to thrive lactosuria (5 25 g/l) sucrosuria (12 g/l) and aminoaciduria Intestinal disaccharidases (including lactase and sucrase) normal at age 6 and 20 weeks Oral lactose tolerance test at this age resulted in lactosuria (4 6 g/l) sucrose tolerance test in sucrosuria (18 5 g/l) In contrast intraduodenal lactose tolerance test gave only low lactose excretion in urine (0 28 g/l) He improved rapidly and had no lactosuria on intraduodenal feeding with citric acid milk The lactosuria diminished as age increased but was still higher at age 6 years than that of controls He tolerated normal disaccharide containing food after 1 5 years of age At 5 5 to 6 years he had symptoms of lactose malabsorption and an isolated lactase deficiency was proved At 10 years he still tolerates only limited amounts of milk The defect in severe familial infantile lactose intolerance seems to be localized in the gastric mucosa Acquired lactase deficiency can appear later in childhood in this syndrome

KEY WORDS Lactose intolerance lactosuria sucrose intolerance sucrosuria disaccharide intolerance lactase deficiency

Durand in 1958 (13) described a condition he called idiopathic lactosuria It has since been called severe infantile familial lactose intolerance and is characterized by a critically ill infant with vomiting and failure to thrive disaccharidurıa and amino aciduria (17 18) The cause of this rare (about 20 cases have been reported) and probably hereditary disorder is still obscure Transient lactase deficiency and increased permeability of the mucosa of the small intestine to lactose has been suggested (6 13 17 18) In a short communication in 1969 we reported normal intestinal lactase activity in a boy in the acute phase of this disorder (4) Moreover no lactosuria occurred when the lactose was given intraduodenally suggesting an increased permeability for lactose in the gastric mucosa

We present here a full account of the studies performed in this boy and a 10-year follow up

METHODS

Lactose tolerance test (LTT) and sucrose tolerance test (STT) were performed after the boy fasted for 6 h (infancy) or overnight (childhood) with a dose of 2 g/kg body weight in 10% solution Glucose–galactose tolerance test (GGTT) was performed with a dose of 1+1 g/kg body weight in 10% solution Intraduodenal LTT Lactose was given through a feeding tube in pars descendens of duodenum under fluoroscopic control

Urine was collected the night before the tolerance tests and 0 to 6 h and 6 to 12 h after The urine was either analysed directly or immediately frozen and stored at − 0°C until analysis

Blood glucose was determined in capillary blood samples taken at 0 15 30 45 and 60 min

Galactose in urine was determined with galactose dehydrogenase (Boehringer A G Mannheim West Germany) (11) as described by Dahlqvist & Svenningsen (10) Glucose in urine was assayed according to Tengstrom (35) The urine was first filtered through a column of ion exchange resin and then analysed with a glucose oxidase reagent Lactose in urine was determined from the increase in galactose after incubation with a fungal lactase (β-galactosidase) 0 5 ml of urine was mixed with 0 5 ml of lactase solution (Maxilact® Gist Brocades Delft The

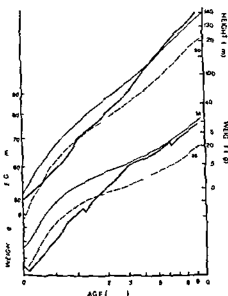


Fig ? Weight and height curves of the patient Mean (*M*) and −3 S D curves of normal children (23) are given for comparison

S-P and S-ALP normal *S urea normal urinary os* molality varied from low to normal

At 17 months he tolerated lactose containing foods at 18 months 300 ml milk per day After age - years he had a normal diet which included up to 1 litre milk/day His weight and height increased and the 50th percentile for weight was reached at 3 years and for height at 4 years (Fig 2)

At 5 5 years he got loose and foul smelling stools Weight standstill Clinitest of faeces was 0 5% Xylose tolerance test was normal (2 0 mmol/l at 30 min and 2 9 mmol/l at 60 min) as was lactose tolerance test (blood glycose increase of 2 mmol/l within 60 min) B folic acid normal Faecal chymotrypsin normal No entero-pathogenic microorganisms in faeces Periodically diarrhoea continued Small intestinal biopsies at 6 and 6 5 years show decreased lactase activity (see below) With a lower lactose intake (about 200 ml milk/day) he became free of symptoms These returned when he drank 4–500 ml milk/day His weight and height were normal (Fig 2) his condition good

SPECIAL INVESTIGATIONS

Urinary excretion of carbohydrates

Table 1 presents the excretion of glucose galactose lactose and sucrose in urine when the patient was on various diets The values from the controls are given as a comparison Large amounts of lactose and sucrose were excreted *during the first month when a lactose or* sucrose containing diet was given The disaccharidura disappeared on a lactose and sucrose free diet The lactosuria reappeared

Table 1 *Urinary excretion of carbohydrates (g/l) in the patient the parents the sister and controls*

	Age	Diet	Glucose	Galactose	Lactose	Sucrose
Patient	1 m	Human milk		0 040	5 ?50	
	? m	Nutramigen			0 080	8 100
	? m	Nutramigen	0 510			11 940
	3½ m	*DSI*	*0 0?8*	*0 010*	0 01?	0
	6 m	*Citrido intraduod*		*0 036*	*0 080*	0 670[a]
	7 m	Citrido per oral		0 0 4	0 1?8	
	8 m	Citrido per oral	0 1?4	0 054	0 540	0 064
	9 m	Findus valling	0 096	0 030	0 164	0 0?4
	10 m	Glucose formula	0 386	0 0 0	0 040	0 012
	1 y	*Glucose formula*		0 010	0 036	
	y	Normal	0 080	0 0?8	0 1?4	
	4 y	Normal	0 046	0 0 ?	0 137	
	6 y	Normal		0 0 0	0 250	
Mother	0 y	Normal		0 040	0 048	
Father	3 y	Normal		0 040	0 048	
Sister	5 d	Human milk		0 157	1 110	
Controls n=9 mean value (range)	1–3 m	Human milk or cow s milk based formula		0 041 (0 016–0 078)	0 ?11 (0 016–0 4?)	

Nutramigen[a]=sucrose containing formula (Mead Johnson) DSI=monosaccharide (glucose) formula (Nestlé) Citrido=citric acid milk Findus välling=cow s milk based formula (AB Findus) Glucose formula=besides the sugar similar to fructose formula (-5)

[a] After 3 ml of a sucrose-containing syrup

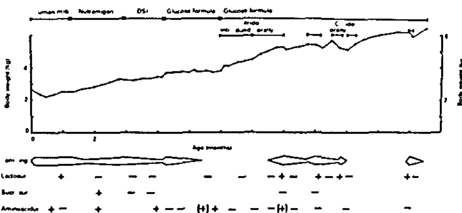


Fig. 1 Survey of the clinical course during the first year of life Nutramigen® = sucrose containing formula (Mead Johnson) DSI = monosaccharide (glucose) formula (Nestle) kindly supplied by Professor B Lindquist Citrido = citric acid milk F V = Findus Valling cow s milk based formula (AB Findus) Glucose formula = apart from the sugar similar to fructose formula (25)

Netherlands) 4 mg/ml in 0 1 mol/l K phosphate buffer pH 6 8) After incubation at 30°C for 1 h the galactose concentration was assayed as described above Sucrose in urine was determined from the increase in glucose after incubation with yeast invertase (sucrase β fructosidase) 0 5 ml of urine was mixed with 0 5 ml of invertase solution (yeast invertase 10 mg/ml in 0 2 mol/l Na acetate buffer pH 4 5 Nutritional Biochem Corp) After incubation for 30 min the glucose concentration was assayed as described above

Paper chromatography of urine The sugars in urine were also identified with semiquantitative paper chromatography As solvent we used ethyl acetate acetic acid water 9 2 2 (deionization of the urine is not needed with this reagent) The spots were developed with aniline phthalate (28) α Amino nitrogen in urine was measured as described by Kachadurian et al (22)

Amino acids in urine were determined with high voltage electrophoresis on paper (16 30)

Other laboratory tests included daily faecal fat determination Clinitest and pH of faeces D-xylose tolerance test trypsin in duodenal juice faecal chymotrypsin sweat test S Ca S P S ALP S ALAT S ASAT S urea and culture of faeces for entero pathogenic microorganisms (for ref see 3) Small intestinal biopsies were taken by a hydraulic capsule at the duodenal flexure under fluoroscopic control (3) Specimens for enzyme analyses were kept at −20°C Specimens for light microscopy were handled as described earlier (3) Gastric mucosal biopsies were taken by the same capsule under fluoroscopic control for light and for electron microscopy

Intestinal disaccharidases were determined according to Dahlqvist (7) and intestinal dipeptidases according to Josefsson & Lindberg (21) and Lindberg et al (24)

Controls consisted of 9 infants 3 weeks to 3 months of age and with the following diagnosis uncomplicated prematurity (2) pyloric stenosis (3) cheilo gnatho palato schisis (1) healthy infants admitted for social reasons or illness of the mother (3)

CASE REPORT

Boy born at term birth weight 2 650 g first child of healthy Swedish parents with a healthy younger sister He was fed human milk but vomited and lost weight He was referred to the Department of Paediatrics because of these symptoms at 10 days of age During the following weeks his main symptoms were excessive vomiting and failure to thrive His stools were normal pH of faeces 6–7 Clinitest of faeces negative X ray of the oesophagus and the stomach at age 2 weeks revealed a slight elongating and narrowing of the pyloric canal At age 5 weeks a second X ray of the stomach and small intestine was normal

Clinitest of urine was positive the sugar was lactose (see below) He had amino aciduria (see below) He began to gain in weight especially when he had a lactose free formula Nutramigen® (Mead Johnson) instead of human milk at 6 weeks of age Fig 1 surveys the clinical course and the various dietary trials during the first year At 5 months of age vomiting stopped when he had a glucose formula (besides the sugar similar to fructose formula (25)) and a saccharose and lactose free diet He got otitis and urinary tract infections (X ray of the urinary tract normal) Weight standstill As he had no symptoms and no lactosuria when the lactose was given intraduodenally (see below) citric acid milk (Citrido) was given intraduodenally (fluoroscopic and X ray control) with a feeding tube from 6 to 7 months of age This resulted in weight gain and he improved clinically he did not vomit and had no lactosuria At 7 months he tolerated increasing amounts of citric acid milk orally At 8 months he had a relapse began to vomit and got lactosuria Lactose and saccharose free diet was given but during the following month he deteriorated got fever and urinary tract infection and excessive vomiting He was dehydrated Fluid was given intravenously for 2 days Then citric acid milk was given intraduodenally for some days resulting in a rapid weight gain Successively clinical improvement at one year of age his weight was 6 2 kg skeletal age delayed he could hardly sit without support his mental development was normal He had no hepato splenomegaly Ophthalmological examination normal Results of various laboratory tests performed during this first year were Faecal fat 2 g/d xylose tolerance test normal (1 9 mmol/l at 30 min and 2 3 mmol/l at 60 min) Lactose tolerance tests normal increase of B glucose (see below) trypsin in duodenal juice normal ×2 (300 and 540 μg/ml) faecal chymotrypsin normal sweat test normal S ASAT and S ALAT normal except for a transitory increase at 5 to 7 months of age S bilirubin normal S Ca

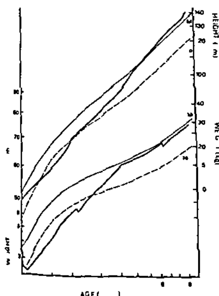


Fig 2 Weight and height curves of the patient Mean (M) and −3 S D curves of normal children (?3) are given for comparison

S-P and S ALP normal S urea normal urinary osmolality varied from low to normal

At 17 months he tolerated lactose-containing foods at 18 months 300 ml milk per day After age ? years he had a normal diet which included up to 1 litre milk/day His weight and height increased and the 50th percentile for weight was reached at 3 years and for height at 4 years (Fig ?)

At 5 5 years he got loose and foul smelling stools Weight standstill Clinitest of faeces was 0 5% Xylose tolerance test was normal (? 0 mmol/l at 30 min and 2 9 mmol/l at 60 min) as was lactose tolerance test (blood glycose increase of 2 mmol/l within 60 min) B folic acid normal Faecal chymotrypsin normal No entero-pathogenic microorganisms in faeces Periodically diarrhoea continued Small intestinal biopsies at 6 and 6 5 years show decreased lactase activity (see below) With a lower lactose intake (about 200 ml milk/day) he became free of symptoms These returned when he drank 4–500 ml milk/day His weight and height were normal (Fig 2) his condition good

SPECIAL INVESTIGATIONS

Urinary excretion of carbohydrates

Table 1 presents the excretion of glucose galactose lactose and sucrose in urine when the patient was on various diets The values from the controls are given as a comparison Large amounts of lactose and sucrose were excreted during the first month when a lactose or sucrose containing diet was given The disaccharidurià disappeared on a lactose and sucrose free diet The lactosuria reappeared

Table 1 *Urinary excretion of carbohydrates (g/l) in the patient the parents the sister and controls*

	Age	Diet	Glucose	Galactose	Lactose	Sucrose
Patient	1 m	Human milk		0 040	5 250	
	? m	Nutramigen			0 080	8 100
	?½ m	Nutramigen	0 510			11 940
	3½ m	DSI	0 0 8	0 010	0 01?	0
	6 m	Citrido intraduod		0 036	0 080	0 670[b]
	7 m	Citrido per oral		0 0?4	0 1?8	
	8 m	Citrido per oral	0 124	0 054	0 540	0 064
	9 m	Findus valling	0 096	0 030	0 164	0 0?4
	10 m	Glucose formula	0 386	0 0?0	0 040	0 012
	1 y	Glucose formula		0 010	0 036	
	y	Normal	0 080	0 0?8	0 124	
	4 y	Normal	0 046	0 02	0 137	
	6 y	Normal		0 0 0	0 50	
Mother	0 y	Normal		0 040	0 048	
Father	3 y	Normal		0 040	0 048	
Sister	5 d	Human milk		0 157	1 110	
Controls n 9 mean value (range)	1 3 m	Human milk or cow s milk based formula		0 041 (0 016–0 078)	0 211 (0 016–0 4?)	

Nutramigen® = sucrose containing formula (Mead Johnson) DSI = monosaccharide (glucose) formula (Nestlé) Citrido citric acid milk Findus välling = cow s milk based formula (AB Findus) Glucose formula = besides the sugar similar to fructose formula (_5)

After 5 ml of a sucrose-containing syrup

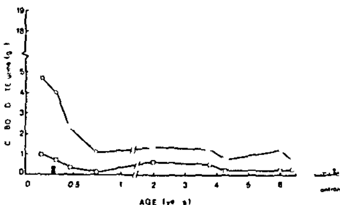


Fig 3 Carbohydrate excretion in urine at lactose and sucrose tolerance tests at various ages of the patient and in the controls (age 1–3 months $n=9$) ○=lactose □=galactose Δ=sucrose ●=lactose intraduodenal LTT ■=galactose intraduodenal LTT

when citric acid milk was given orally at 7 to 8 months of age However the same amounts of citric acid milk given intraduodenally resulted in a considerably lower lactose excretion in urine

The parents had no increase in galactose and lactose in urine His sister at 5 days of age had 1 1 g/l lactose in urine which is within normal limits for this age (5 Dahlqvist & Svenningsen unpublished) Later the Clini test was negative She had no symptoms

Carbohydrate tolerance tests

All carbohydrate tolerance tests gave an increase between 1 2 and 4 95 mmol/l of blood glucose within 60 min (normal >1 1 mmol/l) Urinary excretion of galactose at glucose–galactose tolerance test (GGTT) at 2 months was 1 55 g/l and of glucose 0 4 g/l Fig 3 shows the urinary excretion of lactose and galactose at lactose tolerance tests (LTT) at various ages of the patient and of 8 control infants It is evident that the patient s lactose excretion is high and considerably greater especially at age 2 to 5 months than that of the controls (3 weeks to 3 months of age) In contrast the lactose excretion was low at the intraduodenal LTT at 3 5 months The lactose excretion at intraduodenal LTT was higher in the two controls (3 and 4 weeks of age and prematurely born) The result of their per oral LTT did not differ from those of the other controls The urinary glucose concentration in the various LTT varied between 0 02 to 0 41 g/l At the GGTT and LTT at 2 months of age the boy had a sucrose containing diet (Nutramigen®) The urinary sucrose excretion at these two tests increased from 3 7 to 14 2 g/l and from 6 0 to 13 8 g/l respectively Fig 3 also shows the patient s extremely high sucrose excretion at the sucrose tolerance test (STT) at 2 5 months This contrasts to the low urinary sucrose concentration at STT in the control infant of the same age

There was no immediate clinical reaction at the oral tolerance tests but in infancy the vomiting frequency temporarily increased When older he had no symptoms Intraduodenal LTT gave no reaction

LTT in the parents gave a normal B glucose rise and no abdominal symptoms The father s lactose in urine increased from 0 048 to 0 1 g/l the mother s from 0 048 to 0 096 g/l Corresponding figures for galactose were 0 04 to 0 174 and 0 04 to 0 18 g/l respectively

Amino acid excretion in urine

During the first months he had cystathioninuria All tolerance tests at this age resulted in an increased excretion of cystathionine When he got sucrose containing formula (Nutramigen®) and at the STT he had a generalized amino acidura (α amino nitrogen 700 mg/g creatinine) At the STT the α amino nitrogen increased from 397 to 600 mg/g creatinine After 8 months there were no abnormalities in urinary amino acid excretion The parents had normal amino acid excretion in urine

Small intestinal biopsy

Small intestinal biopsies were taken at 6 and 20 weeks and at 6 and 6 5 years of age The biopsy from 6 weeks showed ridges and leaves and also a small flat area in dissecting microscopy The flat area was almost avillous and showed a heavy plasma cell infiltration (Fig 4*a*) Other areas in the same specimen had a villous mucosa with slightly reduced height



Fig 4 (*a*) Small intestinal biopsy at 6 weeks of age A flat area with elongation of the crypts and infiltration with plasma cells (*b*) Small intestinal biopsy at 6 weeks of age Another part of the biopsy specimen shown in Fig 4*a* Villous structure slight irregularities in surface epithelium (*c*) Small intestinal biopsy at 6 years of age Normal intestinal mucosa

and nuclear irregularities of villous epithelium (Fig 4*b*) The other biopsies showed a normal mucosa (Fig 4*c*) Table 2 shows the intestinal disacchandase activities in the various biopsies The lactase activity was normal in biopsies from 6 and 20 weeks of age but low in biopsies from 6 and 6 5 years The other enzyme activities were within the ranges of the

Table 2 *Intestinal disaccharidase activities in small intestinal biopsies from the patient at various ages*

	6 w	20 w	6 y	6 5 y
Maltase μ/g protein (91–600)	116	51	120	102
Isomaltase μ/g protein (20–126)	46	28	43	33
Sucrase μ/g protein (23–166)	48	29	27 3	25 4
Trehalase μ/g protein (8 3–50)	4 4	11 1	8 6	6 2
Lactase μ/g protein (6 6–73)	15 8	24 8	2 9	3 7

* Controls range (n=49) (8)

controls The intestinal dipeptidase activities on glycyl L leucine L alanyl L glutamic acid L valyl L glutamic acid and L alanyl L proline were analysed in the biopsy from 6 weeks of age and were within normal limits

Gastric biopsy

Light microscopy and electron microscopy of biopsies from fundus and corpus of the stomach taken at 20 and 21 weeks of age showed no abnormalities

DISCUSSION

The main features in severe familial lactose intolerance are the excessive vomiting and the lactosuria during the first months of life These were also our patient s dominant symptoms Moreover he had a pronounced sucrosuria on a diet rich in sucrose This agrees with findings in several other patients (2 6 11 20 29) The lactose and sucrose concentration in urine was occasionally up to 100 times greater than normally found at this age (5) Lactose intolerance is reported to disappear at 6 to 18 months after onset (17) This agrees with our experience The lactosuria in the boy decreased with increasing age as was clearly demonstrated by the lactose excretion in urine at LTT at various ages (see Fig 3) However the lactose concentration in urine at LTT was always higher than those of the controls and also higher than reported in the literature (15) On normal diet from 2 to 6 years of age the lactose concentration in urine was slightly higher than normally found (5)

This pronounced disacchariduria in infancy could be caused by the disaccharides not being hydrolysed because of disaccharidase deficiency but absorbed as such across the small intestinal mucosa However, the patient s lactase and sucrase activities were normal at 6 and 20 weeks of age and also in other patients studied in the acute phase of the disease (1 2 14) Moreover LTT in several patients including the present one (1 2 6 11 19 29) resulted in a normal increase in blood glucose These findings exclude the possibility that the disease is caused by a transient disaccharidase deficiency

The intraduodenal LTT showed that the lactose was split by the lactase in the intestinal mucosa This test gave a normal blood glucose increase and of more interest was not followed by an abnormal lactosuria The urinary lactose concentration was about one twentieth of the concentration when the lactose was given orally and was comparable to that at per oral LTT of the controls (The higher urinary lactose excretion at the intraduodenal LTT in the two controls can be explained by their prematurity and younger age i e the lactase activity not being fully developed) In accordance with the result of the intraduodenal LTT the boy was symptom free improved clinically and had no abnormal lactosuria when cow s milk (citric acid milk) was given intraduodenally between 6 and 7 months of age The symptoms and lactosuria returned when he then had cow s milk orally Russo et al (29) have confirmed these findings At 8 months of age two intraduodenal LTT gave no lactosuria in their patient whereas two oral LTT were followed by lactosuria

Thus it seems that the defect in the gastrointestinal tract is localized more orally than in the duodenum and then most probably in the stomach Light and electron microscopy of

gastric mucosa from the fundus–corpus region was normal The morphology of the antral mucosa was not studied Further studies of the gastric mucosa are needed before reaching any conclusions on the morphology of the gastric mucosa In this context the observations of Moncrieff & Wilkinson (26) and of Woodruff (32) are of interest They found sucrosuria in mentally retarded children who vomited and had hiatus hernia The children also had lac tosuria and one patient vomited excessively and collapsed on lactose (26) Moncrieff & Wilkinson (26) showed that when lactose was given together with sucrose in the diet the sucrosuria became more pronounced this agrees with our findings

The coexistence of pyloric stenosis reported in some infants with severe familial lactose in tolerance (11 20) is difficult to explain The first X ray of our patient s stomach showed slight signs of pyloric stenosis at the second X ray some weeks later these had disappeared Another infant (6) also had slight signs of pyloric stenosis but this could not be veri fied The occurrence of lactosuria in infants with pyloric stenosis has long been known (27) Out of 150 infants with pyloric stenosis Darling et al (11) found reducing substances in urine in one Bichel (5) reported pyloric stenosis among the disorders with excretion of lactose galactose glucose and fructose It can be speculated upon whether the combina tion of a defect in the gastric mucosa as in severe familial lactose intolerance or an irrita tion of the gastric mucosa as in hiatus hernia with an abnormal motility of the stomach can lead to the abnormal absorption of the disac charides in the stomach

The amino aciduria galactosuria and other signs of liver and renal damage are probably secondary phenomena caused by the disac charides (5 6 11)

Interestingly the boy at 5 5 years of age had clinical symptoms of lactose malabsorption Isolated decreased lactase activity was found in two biopsies taken with a 6 month interval and he has still at 10 years of age a limited tolerance for lactose The cause of this acquired lactase deficiency is obscure He had no signs of gastroenteritis of exocrine pan creatic insufficiency or of malnutrition His parents are of Swedish extraction and acquired lactase deficiency at this age in Swedish chil dren is rare (9) Thus a coincidence is not a plausible explanation It has not been reported before in severe familial lactose intolerance but there are no other reports of such a long follow up Holzel (18) reported normal lactase activity after recovery of the acute phase of the disease but his patient was younger at biopsy Russo et al (29) reported from Sicily that four adult male family members had cow s milk intolerance and that 250 ml of cow s milk orally gave each member abdominal pain and lactosuria Two of eight controls got abdominal pain but no lactosuria Their patient and affected family members and also bilateral cataracts not reported earlier in this disease

In conclusion the results from our study in dicate that the basic defect in severe familial lactose intolerance might be localized to the gastric mucosa We suggest that the treat ment in the acute phase is a formula free from disaccharides or intraduodenal feeding with a disaccharide containing formula Lactose mal absorption due to lactase deficiency can de velop later in childhood

REFERENCES

1 Arakawa T Akabane J & Akabene M Lactose intolerance with normal activity of lactase *Tohoku J Exp Med* 83 365 1964
2 Arashima S Matsuda I Sato N & Yoshioka H Intestinal disaccharidase activity in an infant with lac tose in olerance *Helv Paediatr Acta* 26 215 19 1
3 Berg N O Borulf S Jakobsson I & Lindberg T How to approach the child suspected of malabsorption *Acta Paediatr Scand* 67 403 1978
4 Berg N O Dahlqvist A Lindberg T & v Studnitz W Severe familial lactose intolerance—a gastrogen disorder? *Acta Paediat Scand* 58 525 1969
5 Bickel H Melituria a paper chromatographic study *J Pediat* 59 641 1961
6 Carson N A J & Neely R A Disaccharide in tolerance in infancy *Arch Dis Child* 38 574 1963
7 Dahlqvist A Assay of intestinal disaccharidases *Anal Biochem* 22 99 1968

8 Dahlqvist A Lindberg T Meeuwisse G & Åkerman M Intestinal dipeptidases and disaccharidases in children with malabsorption *Acta Paediatr Scand 59* 621 1970
9 Dahlqvist A & Lindquist B Lactose intolerance and protein malnutrition *Acta Paediatr Scand 60* 488 1971
10 Dahlqvist A & Svenningsen N W Galactose in the urine of newborn infants *J Pediatr 75* 454 1969
11 Darling S Mortensen O & Sondergaard G Lactosuria and amino aciduria in infancy *Acta Paediatr Scand 49* 281 1960
12 Duodoroff M D-Galactose dehydrogenase of Pseudomonas saccharophila *In* S P Colowick & N O Kaplan (eds) *Methods in Enzymol* vol 5 Academic Press New York 1962 p 339
13 Durand P Lattosuria idiopatica in una paziente con diarrea cronica ed acidosi *Minerva Pediatr 10* 706 1958
14 Eggermont E & Bracke P Etude clinique et enzymatique de la maladie de Durand XXII Congr de l Association des Pediatres de langue Française Strasbourg 1969
15 Haworth J C Sugars in the blood and urine of children following the ingestion of disaccharides *Arch Dis Child 35* 552 1960
16 Holmgren G Jeppson J O & Samuelsson G High voltage electrophoresis in urinary amino acid screening *Scand J Clin Lab Invest 26* 313 1970
17 Holzel A Sugar malabsorption due to deficiencies of disaccharidase activities and of monosaccharide transport *Arch Dis Child 42* 341 1967
18 Holzel A Disaccharide intolerances *In* K S Holt & V P Coffey (eds) *Some Recent Advances in Inborn Errors of Metabolism* Livingstone Edinburgh 1968 p 101
19 Holzel A Mereu T & Thomson M L Severe lactose intolerance in infancy *Lancet ii* 1346 1962
20 Inall M B & Burkinshaw J H Lactosuria and sucrosuria with failure to thrive *Proc R Soc Med 53* 318 1960
21 Josefsson L & Lindberg T Intestinal dipeptidases I Spectrophotometric determinations and characterization of dipeptidase activity in pig intestinal mucosa *Biochim Biophys Acta 105* 149 1965
22 Kachadurian A Knox W E & Cullen A M Colorimetric ninhydrin method for total alpha amino acids of urine *J Lab Clin Med 56* 321 1960
23 Karlberg P Taranger J Engstrom I Lichtenstein H & Svennberg Redegren I The somatic development of children in a Swedish urban community *Acta Paediatr Scand* Suppl 258 77 1976
24 Lindberg T Norden Å & Josefsson L Intestinal dipeptidases Dipeptidase activities in small intestinal biopsy specimens from a clinical material *Scand J Gastroenterol 3* 177 1968
25 Lindquist B & Meeuwisse G Diets in disaccharidase deficiency and defective monosaccharide absorption *J Am Diet Assoc 48* 307 1966
26 Moncrieff A & Wilkinson R H Sucrosuria with mental defect and hiatus hernia *Acta Paediatr Scand* Suppl *100* 495 1954
27 Rietschel H Inanition und Zuckerausscheidung im Sauglingsalter *Z Kinderheilkd 7* 282 1913
28 Robinsson W M C & Rathbun J C Electrophoresis of free sugar in blood *Science 127* 1501 1958
29 Russo G Mollica F Mazzone D & Santonocito B Congenital lactose intolerance of gastrogen origin associated with cataracts *Acta Paediatr Scand 63* 457 1974
30 Studnitz v W Secondary cystathioninuria *Acta Paediatr Scand 58* 173 1969
31 Tengstrom B Enzymatic determination of glucose and galactose in urine *Scand J Clin Lab Invest 18* Suppl 92 104 1966
32 Woodruff G G Sucrosuria in association with mental deficiency and hiatal hernia *J Pediatr 52* 66 1958

Submitted Dec 14 1978
Accepted Febr 14 1979

(T L) Department of Paediatrics
Malmo General Hospital
S 21401 Malmo
Sweden

SHORT COMMUNICATION

LEAD INTOXICATION AS AN ETIOLOGIC FACTOR IN HYPERKINETIC BEHAVIOR IN CHILDREN A NEGATIVE REPORT

It has been reported that more than half of a group of hyperkinetic children in New York had a high blood lead level (3) Treatment with lead chelating agents improved behavior in these children (4) Several studies have shown that exposure to lead may cause dysfunction in the central nervous system both in man (5 12 13 14 15) and in animals (11) It has also been found that lead intoxication in children is not confined to urban slums but also occurs in smaller communities (7)

If lead intoxication is an important cause of hyperkinetic behavior in children this will have great consequences for therapy as well as for prophylaxis In Scandinavian countries no such investigation has been reported previously In this investigation lead concentration in hair was used as a measure of lead intake since this seems to be a better measure of long term exposure than blood lead level (2 6)

The patients were 19 boys (age 11 9±2 2 years mean ±S D) who had been diagnosed as having a hyperkinetic syndrome Criteria for the diagnosis were the following 1) Motor hyperactivity 2) poor impulse control 3) low frustration tolerance 4) short attention span 5) distractability 6) aggressiveness 7) diminished sensitivity to reinforcement and 8) reduced skin conductance level Details of the diagnostic criteria have been published previously (10)

Twenty two boys (age 11 4±0 7 years) were used as controls Both the patients and the controls lived in the Bergen area (a city of about 210000 inhabitants) however the control group was from a geographically more re[illegible] [illegible] were from the same school Blood lead levels were low in men working in downtown Bergen (1) but high lead concentrations have occasionally been observed in blood hair and teeth in children (8 N P Berg Justesen data to be published)

Samples of 100 mg of hair were obtained from each subject these were taken from the same part of the head and at the same distance from the base of the hair The lead concentration was determined by atomic absorption spectrophotometry as described by others (8 9)

The hair lead concentration in the hyperkinetic group was 7 4±5 4 μg/g (mean ±S D) and in the control group 8 2±7 0 μg/g ($p >$ 0 10 t test) All values observed were well within what is considered to be a normal range (N P Berg Justesen data to be published) The three highest values were in the control group It has been shown that skin conductance level is reduced in children with a hyperkinetic syndrome (10) Correlations between lead concentrations basal skin conductance levels and spontaneous activity (10) were therefore calculated (Pearson product moment correlation) No significant correlations were found either in the hyperkinetic group or in the control group

This investigation indicates that lead intoxication is not an important etiologic factor in hyperkinetic behavior in children in Bergen This of course does not exclude the possibility that lead intoxication may be of importance in areas with more heavy exposure to lead

The authors gratefully acknowledge N P Berg Justesen for valuable suggestions and expert help in performing the lead analyses This research was supported in part by

the Norwegian Research Council for Science and the Humanities and Åndssvakesakens Forskningsfond

K Hole H Dahle H Kløve

Institutes of Physiology and Psychology University of Bergen Årstadveien 19–21 N 5000 Bergen Norway

REFERENCES

1 Bjordal M Romslo I Brinkmann J A & Vollset P Bly og karbonmonoksydnivå i blod hos anleggsarbeidere i Bergen *Tidsskr Nor Lægeforen* 98 886 1978
2 Creason J P Hinners T A Baumgartner J F & Pinkerton C Trace elements in hair as related to exposure in metropolitan New York *Clin Chem 21* 603 1975
3 David O Clark J & Voeller K Lead and hyperactivity *Lancet II* 900 1972
4 David O J Hoffman S P Sverd J Clark J & Voeller K Lead and hyperactivity Behavioral response to chelation A pilot study *Am J Psychiatry 133* 1155 1976
5 de la Burdé B & Choate M S Early asymptomatic lead exposure and development at school age *J Pediatr 87* 638 1975
6 Eatough D J Christensen J J Izatt R M & Hartley C Level of selected trace elements in human hair *In* A C Brown (ed) *The first human hair symposium* Williams & Wilkins Baltimore 1974 p 377
7 Fine P R Thomas C W Suhs R H Cohnberg R E & Flashner B A Pediatric blood lead levels *JAMA 221* 1475 1972
8 Fosse G & Justesen N P Berg Lead in deciduous teeth of Norwegian children *Arch Environ Health 33* 166 1978
9 Harrison W W Yurachek J P & Benson C A The determination of trace elements in human hair by atomic absorption spectroscopy *Clin Chim Acta 23* 83 1969
10 Kløve H & Hole K The hyperkinetic syndrome Criteria for diagnosis *In* R L Trites (ed) *Hyperactivity in children etiology measurements and treatment implications* University Park Press Baltimore 1979 in press
11 Kostas J McFarland D J & Drew W G Lead induced hyperactivity Chronic exposure during the neonatal period in the rat *Pharmacology 14* 435 1976
12 Landrigan P J Whitworth R H Baloh R W Staehling N W Barthel W F & Rosenblum B F Neuropsychological dysfunction in children with chronic low level lead absorption *Lancet I* 708 1975
13 Pihl R O & Parkes M Hair element content in learning disabled children *Science 198* 204 1977
14 Valciukas J A Lilis R Fischbein A Selikoff I J Eisinger J & Blumberg W E Central nervous system dysfunction due to lead exposure *Science 201* 465 1978
15 Waldron H A Lead and human behaviour *J Ment Defic Res 22* 69 1978

Acta Pædiatr Scand 68 761–762 1979

SHORT COMMUNICATION

CONGENITAL TOXOPLASMOSIS LATE APPEARANCE OF RETINAL LESIONS AFTER TREATMENT

Chorioretinitis is frequently observed in congenital toxoplasmosis It is often present at birth but sometimes only develops after several years As far as we know the late appearance of chorioretinitis following treatment of subclinical congenital toxoplasmosis has not been reported

Sophie G a 7 year old girl is the second child of healthy parents During the first hours of life she developed purpura cyanosis hepatosplenomegaly and hypotonia Listeria cytomegalovirus rubella and syphilis were excluded Sabin Feldman and complement fixation tests were strongly positive Toxoplasma was isolated from the patient s CSF after incubation into mouse peritoneum (1) The diagnosis of congenital toxoplasmosis was confirmed by the IgM IFA test (7) Specific treatment consisted of 21 day courses of pyrimethamine (1 mg/kg) and sulfadiazine (100 mg/kg) alternating with spiramycin (50 mg/kg) until the age of 11 months Between birth and 7 1/2 years the optic fundi were repeatedly described as normal by experienced ophthalmologists Neurological and psychomotor development were excellent At the age of seven years bilateral chorioretinitis consistent with the diagnosis of toxoplasmosis was discovered during a routine examination Both fundi showed numerous focal heavily pigmented and sharply demarcated scars In the right eye there were juxta papillary juxta foveal and peripheral lesions In the left eye the lesions were located in the periphery and there was vitreous fibrosis anterior to some of them Physical examination and laboratory data disclosed no other causal agent Neither the toxoplasma serology nor the IgM IFA test supported reinfection by toxoplasmosis The CSF antibody titre (6) appeared equal to that of serum ruling out local C S F production of antibodies due to reactivation of cerebral toxoplasmosis Unfortunately the aqueous humor antibody titre (2) could not be determined A significant rise of ocular antibodies would have been additional proof that the chorioretinitis was due to toxoplasmosis In absence of any signs of reinfection by toxoplasmosis no treatment was instituted

The late appearance of ocular manifestations in congenital toxoplasmosis is well documented (4) As congenital toxoplasmosis is often atypical at birth and because of the late appearance of retinal lesions Desmonts & Couvreur (4) recommend treatment in all newborns suspected of having congenital toxoplasmosis The literature does not mention the appearance of lesions after correct neonatal treatment With regard to the late appearance of retinitis in untreated cases it has been suggested (3) that the lesions are due to a delayed hypersensitivity reaction to the encysted parasites resulting in local proliferation and scar formation Encystation occurs very early after toxoplasmic contamination and while sulfadiazine and pyrimethamine eliminate the trophozoites they do not act on encysted organisms The efficiency of Spiramycin is doubtful and the practical benefit of treatment is still controversial In a 15 year old girl described by Frezotti et al (5) sulfonamides did not prevent worsening of the existing chorioretinitis or extension to the other eye In contrast in eight children with subclinical toxoplasmosis at birth described by Saxon et al (8) only the children who were treated did not develop evidence of intellectual impairment Finally Desmonts (3) has confirmed our ob-

servation and stated that treatment only reduces the frequency of retinopathy but cannot completely prevent it

We are indebted to Dr Desmonts (Lab de sérologie neonatale et de recherche sur la toxoplas a Paris) for his helpful advice

M De Vroede *A Piepsz*
J Dodion *C Verougstraete*
F De Meuter

(M D V) Department of Paediatrics
St Pieters Ziekenhuis
Hoogstraat 320
B 1000 Brussels
Belgium

REFERENCES

1 De Meuter F La toxoplasmose en Belgique *Mem Acad R Med Belg* 2ème série 5 163 1967
2 Desmonts G Toxoplasmose oculaire Etude epidemiologique *Arch Ophtalmol* (Paris) *33* 87 1973
3 Desmonts G Personal communication
4 Desmonts G & Couvreur J Congenital toxoplasmosis A prospective study of 378 pregnancies *N Engl J Med 290* 1110 1974
5 Frezotti R Guerra R Terragna A & Tosi P A case of congenital toxoplasmosis with active chorioretinitis *Ophtalmologica 169* 321 1974
6 Pussan C Desmonts G & Mozzioconacci P Evolutivité neurologique tardive d une toxoplasmose congenitale démontrée par l étude du L C R *Ann Pediatr 18* 224 1971
7 Remington J S Miller M J & Brownlee I IgM antibodies in acute toxoplasmosis II Prevalence and significance in acquired cases *J Lab Clin Med 71* 855 1968
8 Saxon S A Knight W Reynolds D W Stagno S & Alford C A Intellectual deficits in children born with subclinical congenital toxoplasmosis *J Pediatr 82* 792 1973

Acta Pædiatr Scand 68 763–764 1979

SHORT COMMUNICATION

REGRESSION OF WILMS TUMOR AFTER PREOPERATIVE CHEMOTHERAPY

In all cases of Wilms tumor two-year survival rates of more than 80% can now be expected after the combined use of surgery radiation and chemotherapy (1 3 4 5 6 8) However the best way of using and combining these three treatments is still a matter of controversy In studies initiated by and under the surveillance of the International Society of Pediatric Oncology (SIOP) studies concerning the effect of preoperative chemotherapy were started in the beginning of the 70 s Preliminary results were published in 1976 (8) It was concluded that pre operative radiation therapy prevented rupture of the tumor at operation which seemed to be correlated to a higher recurrence free survival In a later study to be published by SIOP preoperative radiation was combined with preoperative chemotherapy Nephrectomy was followed by multiple courses of combined actinomycin D and vincristine the latter therapeutic regime originally suggested by D Angio et al (2) The results in this SIOP study appear to be superior to that in the earlier SIOP study However preoperative radiation may have late side effects Therefore it might be preferable to omit preoperative radiotherapy in favour of preoperative chemotherapy alone Sullivan et al (9) had already by 1967 replaced preoperative radiation with chemotherapy alone and had shown marked effects on the tumors Therefore in 1977 SIOP initiated a prospective study to investigate whether pre-operative chemotherapy alone without radiation can maintain the good results regarding tumor rupture at operation and the overall long term survival The outcome of this study in which we are taking part will of course not be available in several years We have however observed a remarkable regression of Wilms tumor after preoperative chemotherapy alone in our first four consecutive patients and therefore a preliminary report seems indicated

The patients were 6 months 2 2 5 and 7 5 years respectively The preoperative treatment consisted of intravenously administered actinomycin D and vincristine over a period of three weeks Actinomycin D was given in a dose of 15 μg/kg body weight on day 1 2 3 14 16 and 17 and vincristine in a dose of 1 5 mg/kg body weight on day 1 8 15 and 22 Angiography was performed both before treatment started and on the day of operation which was scheduled one week after the chemotherapy was completed Selective angiography was performed via catheterization of the renal artery as well as other aortic branches that might supply the tumor At initial angiography vascular supply from the renal artery was graded as gross in three cases and moderate in one Two tumors also had a moderate vascular supply from lumbar arteries Angiography after chemotherapy demonstrated a remarkable decrease in tumor vascularity in all children and in two tumor vessels were completely absent There was a marked reduction in the bulk of the tumor in all cases In 3 out of the 4 children angiography after chemotherapy demonstrated considerably more intact renal parenchyma than before treatment At nephrectomy slight perirenal oedema was found but no fibrosis Three of the kidneys were easily removed but one largely necrotic tumor ruptured According to the SIOP staging classification of Wilms tumor two of the tumors were stage I (one

highly and one moderately differentiated) one stage II and one stage III (both moderately differentiated) The histological classification was that of Jereb & Sandstedt (7) All the tumors showed large necrotic areas with vital tumor tissue generally present only in the periphery

The effect of preoperative chemotherapy treatment on the tumor was well demonstrated at the sequential angiographic studies The degree of reduction of blood supply indicated an extensive devitalisation of tumor tissue Histological examination confirmed that all tumors showed widespread necrosis The effect on the tumor was independent of its degree of differentiation In the two tumors where angiography demonstrated vascular supply from lumbar arteries pericapsular growth could be shown The angiography after chemotherapy demonstrated in addition to the regression of the tumor tissue considerably more renal parenchyma than before treatment This might be due to a recovery of the renal parenchyma when the growing tumor begins to shrink

The present preliminary report may suggest that preoperative radiotherapy can be changed to chemotherapy alone in the treatment of Wilms tumor

B Jacobsson *S E Sorensen*
A Rubenson *S Hagberg*
G Hanson

(A R) Department of Surgery
Children s Hospital
Östra Sjukhuset
S 41685 Goteborg
Sweden

REFERENCES

1 Cassady J D Tefft M Filler R M Jaffe N & Hellman S Considerations in the relation therapy of Wilms tumor *Cancer 32* 598 1973
2 D Angio G J Beckwith J B Bishop H C Breslow N Evans A Goodwin W King L Pickett L Sinks L F Sutow W & Wolff J The National Wilms Tumor Study a progress report *Proc Natl Cancer Conf 7* 627 1973
3 D Angio G J Beckwith J B Bishop H C Breslow N Feigl P Evans A Goodwin W Pickett L Sinks L F Sutow W Tefft M & Wolff J The National Wilms Tumor Study (NTWS)—Preliminary results *Proc Am Assoc Cancer Res 15* 68 1974 (Abstr)
4 D Angio G J Evans A E Breslow N Beckwith J B Bishop H Feigl P Goodwin W Leape L L Sinks L F Sutow W Tefft M & Wolff J The treatment of Wilms tumor *Cancer 38* 633 1976
5 Farber S Chemotherapy in the treatment of leukemia and Wilms tumor *JAMA 198* 826 1966
6 Jenkin D & Sonley M Wilms tumor—Treatment results 1969–73 Implications for the future *Proc Am Soc Clin Oncol 16* 239 1975
7 Jereb B & Sandstedt B Structure and size versus prognosis in nephroblastoma *Cancer 31* 1471 1973
8 Lemerle J Voute P A Tournade M F Delemarre J F M Jereb B Åhstrom L Flamant R & Gerard Marchant R Preoperative versus postoperative radiotherapy single versus multiple courses of actinomycin D in the treatment of Wilms tumor *Cancer 38* 647 1976
9 Sullivan M P Sutow W Cangie A & Taylor G Vincristine sulfate in management of Wilms tumor Replacement of preoperative irradiation by chemotherapy *JAMA 202* 381 1967

Acta Pædiatr Scand 68 765–767 1979

CASE REPORT

PNEUMONIA ASSOCIATED WITH *CHLAMYDIA TRACHOMATIS* INFECTION IN AN INFANT

ANITA HALLBERG PER ANDERS MÅRDH KENNETH PERSSON and TORVALD RIPA

From the Department of Paediatrics University of Lund Malmo General Hospital Malmo the Department of Medical Microbiology University of Lund Lund and the Department of Virology University of Lund Malmo General Hospital Sweden

ABSTRACT Hallberg A Mårdh P A Persson K and Ripa T (Departments of Paediatrics and Virology Malmo General Hospital and Department of Medical Microbiology University Hospital Lund Sweden) Pneumonia associated with *Chlamydia trachomatis* infection in an infant Acta Paediatr Scand 68 765 1979.—*Chlamydia trachomatis* was isolated from the epipharynx of a 10-week-old baby girl taken ill with pneumonia but without signs of conjunctivitis The infant developed specific antibodies to the organism The course of the pneumonia was protracted with cough and tachypnea The baby who was afebrile improved on antibiotic therapy but pulmonary infiltrates persisted for several months To our knowledge this is the first case of pneumonia in an infant associated with *C trachomatis* infection reported elsewhere than North America

KEY WORDS Infant *Chlamydia trachomatis* pneumonia

During recent years it has become evident that *Chlamydia trachomatis* is a common cause of sexually transmitted diseases (15) and complications thereof such as acute epididymitis (5) and salpingitis (11)

C trachomatis may be transmitted from the mother to her baby resulting in conjunctivitis (12) and pneumonia (3) The first case of pneumonitis associated with *C trachomatis* infection was first described in 1975 by Schachter and colleagues (14) Since then a number of reports have appeared (2 3 7 8 9 10 15) all from North America describing chlamydia associated pneumonitis in newborns

In the present communication a case of pneumonia strongly suspected to be caused by *C trachomatis* is described in a Swedish infant

CASE HISTORY

A 10-week-old girl was admitted to the Department of Paediatrics Malmö General Hospital because of wheezing and cough persisting for 2 weeks She was in good condition and afebrile A diagnosis of obstructive bronchitis was recorded Two weeks later she was brought back with symptoms of tachypnea cough and wheezing but was still afebrile A pulmonary radiograph revealed diffuse interstitial and patchy alveolar infiltrates She was given ampicillin (50 mg/kg/day) for 6 days and the symptoms regressed

C trachomatis was isolated from the epipharynx by means of an isolation technique described elsewhere (13) Tests for *Bordetella pertussis* and cytomegalovirus proved negative No antibodies to cytomegalovirus respiratory syncytial virus or to *Mycoplasma pneumoniae* were found

By the time the result of the culture for chlamydia was available she had improved and another pulmonary radiograph showed only a few remaining infiltrates Nevertheless erythromycin (60 mg/kg/day) was given for 2 weeks One week later the infant again had diffuse and wide spread lung infiltrates and erythromycin was given for a further ? weeks One week after the conclusion of this treatment the patient began to cough again and the lung infiltrates had progressed Trimethoprim/sulphamethoxazole (trimethoprim 8 mg/kg/day and sulphamethoxazole 40 mg/kg/day) was then given for 2 weeks after which her symptoms and the infiltrates disappeared Five days after she had completed the treatment course the infant once again started to cough she had tachypnea and was wheezing She was then admitted to the Department of Paediatrics

C trachomatis could no longer be isolated form the epipharynx nor from rectum vagina or the conjunctivae There was no eosinophilia and the serum levels of IgG and IgM were within the normal range (Table I) The first serum sample from the infant was obtained at the age of 5 ½ months IgG (but not IgM) antibodies to *C trachoma*

Table 1 *Results of some laboratory tests*
NT = not tested

Parameter studied	Age (weeks) 12	17	22
S Leukocytes (×10⁹/l)	6.0	9.0	7.8
ESR (mm)	25	18	13
S Eosinophils (×10⁶/l)	NT	NT	187
P IgG (g/l)	NT	NT	7
P IgM (g/l)	NT	NT	0.8
Weight (g)	4 890	5 620	6 470
Rectal temp (°C)	37.2	NT	36.8

tis at a titre of 1 512 were demonstrated using a micro-immunofluorescence test (17)

Trimethoprim/sulphamethoxazole was given for a further 4 weeks The treatment resulted in the disappearance of the symptoms but diffuse pulmonary infiltrates still persisted

At delivery the mother had IgG antibodies against *C trachomatis* at a titre of 1 16–1 32 Six months later the titre was 1 256 (IgG) At the time when the girl was admitted to the Paediatric Department *C trachomatis* could not be isolated from the cervix/urethra of the mother However 3 months later the organism was recovered from both these sites *C trachomatis* could not be isolated from the single urethral specimen obtained from the father

DISCUSSION

Infants may after birth be colonized with *C trachomatis* in the eyes and/or epipharynx as well as in the rectum and genital tract (3 14 15) Some colonized infants may develop conjunctivitis and/or pneumonia Recently Schachter et al (16) estimated that 14 out of 1 000 newborns in San Francisco develop conjunctivitis due to an infection with *C trachomatis* The corresponding figure for pneumonia is 8 Harrison and co workers (10) found *C trachomatis* in the nasopharynx and/or a specific antibody response to the organism in 9 out of 30 infants with signs of pneumonitis and in one of the 28 matched controls Beem & Saxon (3) reported that in 18 infants with *C trachomatis* associated pneumonia only half had conjunctivitis They also found that 10 of 11 infants with conjunctivitis but without lower respiratory tract illness had a positive culture for *C trachomatis* from the nasopharynx (3)

C trachomatis was isolated from the epipharynx of our patient whereas cultures from both eyes proved negative Two months after the isolation of *C trachomatis* from the epipharynx the child still had signs of pneumonia She then had IgG antibodies to *C trachomatis* at a titre of 1 512 There is no reason to believe that the antibodies demonstrated in the infant originated from the mother as the level in the child was much higher than had been detected in the mother prior to birth The high level of IgG antibodies in the child was found at the age of 5½ months when maternal antibodies would be virtually gone The failure to detect IgM antibodies probably reflects the late timing of specimen collection

Conclusive evidence of *C trachomatis* as being the cause of pneumonitis in newborn infants is the isolation of the organism from open lung biopsies of children with pneumonitis who have developed high titres of specific antibodies to the organism (2 8) In our case the organism was not isolated directly from the pulmonary lesions but circumstantial evidence strongly points to *C trachomatis* as being the cause of the pneumonia The clinical course was consistent with that of some of the earlier reported cases of *C trachomatis* associated pneumonia in infants (3 9 10 14 15)

C trachomatis has been recovered in several series of pregnant women in frequencies up to 12% (1 6 15) It has been estimated (1 6 15) that the risk of a child developing a clinical infectoin with *C trachomatis* is at least 50% if borne by a woman infected with the organism in the lower genital tract The first set of cultures for *C trachomatis* from the mother in the case described were negative which however might be due to the fact that the specimens had been stored frozen prior to study

Harrison et al (10) found that eosinophilia and/or elevated serum levels of IgG and IgM occur in some newborns with pneumonitis caused by *C trachomatis* No serum specimens were available from our patient until she reached the age of 5½ months However on this occasion the infant had no eosinophilia

and normal serum levels of IgG and IgM (Table 1)

The protracted course of the disease in our patient corresponds to some earlier described cases (2 3) It may be wondered whether the child was re infected by her mother who re ceived no treatment until late in the course of her baby s illness

Beem & Saxon (4) administered sulphisoxa zole (150 mg/kg/day) to some infants with pneumonia and *C trachomatis* infection while erythromycin ethylsuccinate (40 mg/kg/day) was given to others Four days after treat ment with either of these antibiotics chla mydiae could no longer be recovered from the epipharynx of the patients but not until 3 weeks later did pulmonary radiographs appear normal In other cases of *C trachomatis* associated pneumonia (9) recovery after 3 months without the institution of antibiotic treatment has been described However as some infants may be seriously ill systemic therapy with erythromycin or sulphonamides has been recommended (4 15) The infant de scribed here was given erythromycin for two 2 week periods and trimethoprim/sulpha methoxazole for a further 2 and 4 weeks respectively During treatment she improved but the symptoms reappeared and the pul monary infiltrates progressed shortly after treatment was discontinued This indicates that antibiotic treatment of infants with pneu monia caused by *C trachomatis* should be ad ministered over a comparatively long period of time

ACKNOWLEDGEMENT

This study was supported by grant 16X-4509 by the Swedish Medical Research Council

REFERENCES

1 Alexander E R Chandler J W Pheiffer T A Wang S P English M & Holmes K K Prospective study of perinatal *Chlamydia trachomatis* infection *In* D Hobson & K K Holmes (eds) *Nongonococcal urethritis and related infections* American Society for Microbiology Washington D C 1977 p 148

2 Arth C Von Schmidt B Grossman M & Schachter J Chlamydial pneumonitis *J Pediatr 93* 447 1978

3 Beem M O & Saxon E M Respiratory tract colonization and a distinctive pneumonia syndrome in infants infected with *Chlamydia trachomatis N Engl J Med 296* 306 1977

4 Beem M O & Saxon E M Uncertainties in the treatment of Chlamydia infections in infants *N Engl J Med 296* 1174 1977

5 Berger R E Alexander E R Monda G D Ansell J McCormick G & Holmes K K *Chlamydia trachomatis* as a cause of acute idiopathic epididymitis *N Engl J Med 298* 301 1978

6 Chandler J W Alexander E R Pheiffer T A Wang S P Holmes K K & English M Ophthalmia neonatorum associated with maternal chlamydial infections *Trans Am Acad Ophthalmol Otolaryngol 83* 302 1977

7 Embil J A Ozere R L & MacDonald S W Chlamydia trachomatis and pneumonia in infants report of two cases *Canad Med Assoc J 119* 1199 1978

8 Frommell G T Bruhn F W & Schwartzman J D Isolation of *Chlamydia trachomatis* from infant lung tissue *N Engl J Med 296* 1150 1977

9 Hammerschlag M R Chlamydial pneumonia in infants *N Engl J Med 298* 1083 1978

10 Harrison H R English M G Lee C K & Alexander E R *Chlamydia trachomatis* infant pneumonitis *N Engl J Med 298* 702 1978

11 Mårdh P A Ripa K T Svensson L & Westrom L *Chlamydia trachomatis* infection of the fallopian tubes in patients with acute salpingitis *N Engl J Med 296* 1377 1977

12 Rees E Tait A Hobson D & Johnson F W A Chlamydia in relation to cervical infection and pelvic inflammatory disease *In* D Hobson & K K Holmes (eds) *Nongonococcal urethritis and related infections* American Society for Microbiology Washington D C 1977 p 67

13 Richmond S J Isolation of chlamydia subgroup A (*Chlamydia trachomatis*) in irradiated McCoy cells *Medical Laboratory Technology 31* 7 1974

14 Schachter J Lum L Gooding C A & Ostler B Pneumonitis following inclusion blennorrhea *J Pediatr 87* 779 1975

15 Schachter J Chlamydia infections *N Engl J Med 298* 478 490 and 540 1978

16 Schachter J Grossman M Holt J Sweet R & Mills J Prospective study on chlamydial infection in the newborn *18th Interscience Conference on Antimicrobial Agents and Chemotherapy* Atlanta Ga 1978 Abstract No 477

17 Treharne J D Darougar S & Jones B R Modification of the micro-immunofluorescence test to provide a routine serodiagnostic test for chlamydial infection *J Clin Pathol 30* 510 1977

Submitted Jan 16 1979
Accepted March 5 1979

(A H) Department of Paediatrics
Malmö General Hospital
S-21401 Malmö
Sweden

Table I. *Results of some laboratory tests*
NT = not tested

Parameter studied	Age (weeks) 12	17	22
S Leukocytes (×10⁹/l)	6.0	9.0	7.8
ESR (mm)	25	18	13
S Eosinophils (×10⁶/l)	NT	NT	187
P IgG (g/l)	NT	NT	7
P IgM (g/l)	NT	NT	0.8
Weight (g)	4 890	5 670	6 470
Rectal temp. (°C)	37.2	NT	36.8

tis at a titre of 1:512 were demonstrated, using a micro-immunofluorescence test (17).

Trimethoprim/sulphamethoxazole was given for a further 4 weeks. The treatment resulted in the disappearance of the symptoms but diffuse pulmonary infiltrates still persisted.

At delivery, the mother had IgG antibodies against *C. trachomatis* at a titre of 1:16–1:32. Six months later the titre was 1:256 (IgG). At the time when the girl was admitted to the Paediatric Department, *C. trachomatis* could not be isolated from the cervix/urethra of the mother. However, 3 months later, the organism was recovered from both these sites. *C. trachomatis* could not be isolated from the single urethral specimen obtained from the father.

DISCUSSION

Infants may after birth be colonized with *C. trachomatis* in the eyes and/or epipharynx as well as in the rectum and genital tract (3, 14, 15). Some colonized infants may develop conjunctivitis and/or pneumonia. Recently Schachter et al. (16) estimated that 14 out of 1 000 newborns in San Francisco develop conjunctivitis due to an infection with *C. trachomatis*. The corresponding figure for pneumonia is 8. Harrison and co-workers (10) found *C. trachomatis* in the nasopharynx and/or a specific antibody response to the organism in 9 out of 30 infants with signs of pneumonitis and in one of the 28 matched controls. Beem & Saxon (3) reported that in 18 infants with *C. trachomatis* associated pneumonia, only half had conjunctivitis. They also found that 10 of 11 infants with conjunctivitis but without lower respiratory tract illness had a positive culture for *C. trachomatis* from the nasopharynx (3).

C. trachomatis was isolated from the epipharynx of our patient, whereas cultures from both eyes proved negative. Two months after the isolation of *C. trachomatis* from the epipharynx the child still had signs of pneumonia. She then had IgG antibodies to *C. trachomatis* at a titre of 1:512. There is no reason to believe that the antibodies demonstrated in the infant originated from the mother, as the level in the child was much higher than had been detected in the mother prior to birth. The high level of IgG antibodies in the child was found at the age of 5 1/4 months when maternal antibodies would be virtually gone. The failure to detect IgM antibodies probably reflects the late timing of specimen collection.

Conclusive evidence of *C. trachomatis* as being the cause of pneumonitis in newborn infants is the isolation of the organism from open lung biopsies of children with pneumonitis who have developed high titres of specific antibodies to the organism (2, 8). In our case the organism was not isolated directly from the pulmonary lesions but circumstantial evidence strongly points to *C. trachomatis* as being the cause of the pneumonia. The clinical course was consistent with that of some of the earlier reported cases of *C. trachomatis* associated pneumonia in infants (3, 9, 10, 14, 15).

C. trachomatis has been recovered in several series of pregnant women in frequencies up to 12% (1, 6, 15). It has been estimated (1, 6, 15) that the risk of a child developing a clinical infectoin with *C. trachomatis* is at least 50% if borne by a woman infected with the organism in the lower genital tract. The first set of cultures for *C. trachomatis* from the mother in the case described were negative, which, however, might be due to the fact that the specimens had been stored frozen prior to study.

Harrison et al. (10) found that eosinophilia and/or elevated serum levels of IgG and IgM occur in some newborns with pneumonitis caused by *C. trachomatis*. No serum specimens were available from our patient until she reached the age of 5 1/2 months. However, on this occasion the infant had no eosinophilia

Acta Pædiatr Scand 68 769–771 1979

CASE RFPORT

THE 49 XXXXX SYNDROME

Report of a Case with 48 XXXX/49 XXXXX Mosaicism

M CIRILLO SILENGO G F DAVI and P FRANCESCHINI

From the Clinical Genetics Center First Paediatric Clinic
University of Turin Torino Italy

ABSTRACT Cirillo Silengo M Davi G F and Franceschini P (the Clinical Genetics Center First Paediatric Clinic University of Turin Turin Italy) The 49 XXXXX syndrome Acta Paediatr Scand 68 769 1979.—A patient with 48 XXXX/49 XXXXX mosaicism is presented Clinical findings include severe growth and developmental retardation hyper telorism mongoloid slant of the palpebral fissures clinodactyly of the fifth fingers retarded bone age and radio-ulnar synostosis The findings are similar to those of the cases with a penta X chromosome complement already described and are also similar to the signs of the more common 49 XXXXY syndrome of males In both instances the dysmorphic features are less impressive than the mental retardation and the skeletal malformations This report contributes to a better delineation of the 49 XXXXX syndrome The possible mechanisms of the chromosomal aberration are discussed

KEY WORDS Chromosomal aberration X polisomy malformation syndromes

Hitherto only 8 patients with a 49 XXXXX karyotype have been reported by Berger et al (1) Borbolla Vacher et al (2) Brody et al (3) Giovannucci Uzielli et al (6) Kaufman et al (9) Kesaree & Wooley (10) Larger Piet et al (11) Sergovich et al (13) and Yamada & Nenishi (14)

Two 48 XXXX/49 XXXXX mosaics were described by Cooke et al (4) and by Dalla piccola & Pistocchi (5) The patient described by Gordon & Paulsen (7) had a complex mo saicism with four cell lines (XO/XX/XXX/XXXX)

We describe here a 14-month old female with a 48 XXXX/49 XXXXX mosaicism who has clinical and radiological findings similar to those of the previously described cases

CASE REPORT

R M female age 14 months

Both parents are 3 years old and healthy A 7 year-old brother is normal She was the full term product of an un ...y Delivery was accomplished by cesarean section because of uterine inertia Birth weight 1900 g She was hypertonic as a newborn and failed to thrive The psychomotor development has been delayed

Physical examination

Weight 5300 g height 80 cm head circumference 4? cm She has a peculiar facies with frontal bossing hyper telorism upward slant of the palpebral fessures and simple pattern ears (Fig 1)

She has marked clinodactyly of the fifth fingers Der matoglyphics show bilateral t displaced axial triradii and hypothenar loops Because of the extreme dermal ridge hypoplasia the fingerprint pattern could not be evaluated

She has hypotonia and joint hyperflexibility Her devel opment is severely retarded she has poor head control cannot sit up stand walk nor talk

Laboratory Studies

Routine investigations are within normal limits The radio logical findings are marked hypoplasia of the middle phalanx of the fifth fingers producing clinodactyly (Fig 2) proximal radio-ulnar synostosis (Fig 3) and increased iliac angle The bone age is that of a newborn The intra venous urogram is normal

Cytogenetic Studies

The chromosomal analysis of the blood lymphocytes is consistent with a mosaicism with two cell lines one with 48 chromosomes (25%) and one with 49 chromosomes

tion by a normal X bearing sperm This hypothesis is based upon the data from the Xg blood group studies in three cases of X polisomy in whom the maternal derivation of the X s was demonstrated and mosaicism was excluded (8 12) In our patient who has 48 XXXX/49 XXXXX cell lines one X chromosome could have been lost in a mitotic division of an originally 49 XXXXX zygote The presence of only two cell lines is against the hypothesis of repeated non disjunctional events in mitotic divisions of a normal XX or a XXX zygote This is probably the more reasonable interpretation of the patient reported by Gordon & Paulsen (7) who had four cell lines with a XO/XX/XXX/XXXXX mosaicism

In our case the Xg blood group was not informative On the other hand those studies when contributory allow us to confirm or to disprove the paternal contribution of X chromosomes but do not demonstrate whether the non disjunctional events occur in meiosis post zygotic mitosis or both The presence of multiple cell lines seems a better criterion by which to ascertain the mitotic origin of a non disjunctional error

REFERENCES

Berger R Loewe Lyon S Derre J & Ortiz M A Syndrome 49 XXXXX *Ann Pediatr 20* 965 1973

Borbolla Vacher L Vasqez Rios B Pozo Lauzan D & Alvarez Mesa S Sindrome 49,XXXXX Presentacion de un caso *Rev Cub Pediatr 47* 4 1975

Brody J Fitzgerald M C & Spies A S A female child with five X chromosomes *J Pediatr 70* 105 1967

Cooke P Blanck J A & Curtid D J Comparative clinical studies and X chromosome behaviour in a case of XXXX/XXXXX mosaicism *J Med Genet 9* 235 1972

Dallapiccola B & Pistocchi G F Alterazioni scheletriche in un caso di rara aberrazione cromosomica (paziente a corredo cromosomico 48 XXXX/49 XXXXX) studio comparativo con la sindrome 49 XXXXY e la sindrome 49 XXXXX *Radiol Med 54* 737 1968

Giovannucci Uzielli M L Torricelli F Salvatori Q Consumi I Donzelli G & Seminara S Corredo cromosomico 49 XXXXX in una bambina con ipoevolutismo psicofisico *Minerva Pediatr 27* 2270 1975

Gordon D L & Paulsen C A Premature menopause in XO/XX/XXX/XXXXX mosaicism *Am J Obstet Gynecol 97* 85 1967

Grouchy de J Brissaud H E Richardet J M Repesse G Sanger R Race R R Salmon C & Salmon D Sindrome 48 XXXX chez une enfant de six ans Transmission anormale du groupe Xg *Ann Genet 11* 120 1968

Kaufman L Sekhon G S Brazy J E Sivakoff M C & Hatahet G 49 XXXXX syndrome in a neonate *Birth Defects 11* (5) 333 1975

Kesaree L & Wooley P V A phenotypic female with 49 chromosomes presumably XXXXX *J Pediatr 63* 1099 1963

Larger Piet A Bertholot J & Emerit I Une observation du sindrome 49 XXXXX *Nouv Presse Med 1* 467 1972

Lewis F J W Froland A Sanger R & Race R R Source of the X chromosome in two XXXXY males *Lancet ii* 1099 1964

Sergovich F Uilenber C & Pozsonli J The 49 XXXXX chromosome constitution similarity to the 49 XXXXY condition *J Pediatr 78* 285 1971

Yamada Y Nenishi S Penta X (49 XXXXX) chromosome constitution a case report *Jap J Hum Genet 16* 15 1971

Submitted May 22 1978
Accepted Febr 19 1979

(P F) I Pediatric Clinic
University of Turin
Piazza Polonia 94
10126 Torino
Italy



Fig 1 Patient s phenotype

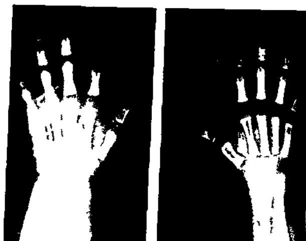


Fig 2 Hands X rays illustrating the clinodactyly of the fifth fingers

(75%) The extra chromosomes were identified as X s with Q bands by fluorescence using quinacrine and with G bands by trypsin using Giemsa

The buccal smear is positive in 84% of the examined cells 15% of the cells have a single Barr body Two three and four Barr bodies are present in 23% of the cells respectively Skin fibroblasts have the same chromosomal pattern Thus the patient is a 48 XXXX/49 XXXXX mosaic The karyotype of both parents is normal The Xg blood group study is non contributory

DISCUSSION

On comparing the literature data with ours the existence of a penta X syndrome seems acceptable

Constant findings are severe psychomotor retardation growth failure peculiar facies with coarse features hypertelorism mongoloid slant of the palpebral fissures clinodactyly of the fifth fingers Half of the patients have retarded bone age and radio ulnar synostosis In three cases (2 5 13) a number of other skeletal malformations similar to those found in the 49 XXXXY are recorded

The clinical similarity of males and females with 49 chromosomes and to a lesser extent females with 48 XXXX karyotype draws attention to the possible effect of constitutive heterochromatin overdosage in skeletal and mental development as well as in phenotype expression The marked prevalence of 49 XXXXY males over 49 XXXXX and 48 XXXX females is possibly explained as a bias of ascertainment due to the genital anomalies in males

The simplest explanation for a 49 XXXXX karyotype is a double meiotic non disjunction in the mother with the production of a 26 XXXX ovum and its subsequent fecunda



Fig 3 Elbow X rays bilateral radio ulnar synostosis

tion by a normal X bearing sperm This hypothesis is based upon the data from the Xg blood group studies in three cases of X polisomy in whom the maternal derivation of the X s was demonstrated and mosaicism was excluded (8 12) In our patient who has 48XXXX/49XXXXX cell lines one X chromosome could have been lost in a mitotic division of an originally 49XXXXX zygote The presence of only two cell lines is against the hypothesis of repeated non disjunctional events in mitotic divisions of a normal XX or a XXX zygote This is probably the more reasonable interpretation of the patient reported by Gordon & Paulsen (7) who had four cell lines with a XO/XX/XXX/XXXXX mosaicism

In our case the Xg blood group was not informative On the other hand those studies when contributory allow us to confirm or to disprove the paternal contribution of X chromosomes but do not demonstrate whether the non-disjunctional events occur in meiosis post zygotic mitosis or both *The presence of multiple cell lines seems a better criterion by which to ascertain the mitotic origin of a non disjunctional error*

REFERENCES

Berger R Loeve Lyon S Derre J & Ortiz M A Syndrome 49 XXXXX *Ann Pediatr* 20 965 1973

Borbolla Vacher L Vasqez Rios B Pozo Lauzan D & Alvarez Mesa S Sindrome 49 XXXXX Presentacion de un caso *Rev Cub Pediatr* 47 4 1975

Brody J Fitzgerald M C & Spies A S A female child with five X chromosomes *J Ped at* 70 105 1967

Cooke P Blanck J A & Curtid D J Comparative clinical studies and X chromosome behaviour in a case of XXXX/XXXXX mosaicism *J Med Genet* 9 235 1972

Dallapiccola B & Pistocchi G F Alterazioni scheletriche in un caso di rara aberrazione cromosomica (paziente a corredo cromosomico 48XXXX/49XXXXX) studio comparativo con la sindrome 49XXXXY e la sindrome 49XXXXX *Radiol Med* 54 737 1968

Giovannucci Uzielli M L Torricelli F Salvatori Q Consumi I Donzelli G & Seminara S Corredo cromosomi o 49 XXXXX in una bambina con ipoevolutismo psicofisico *Minerva Pediatr* 27 2720 1975

Gordon D L & Paulsen C A Premature menopause in XO/XX/XXX/XXXXX mosaicism *Am J Obstet Gynecol* 97 85 1967

Grouchy de J Brissaud H E Richardet J M Repesse C Sanger R Race R R Salmon C & Salmon D *Sindrome 48 XXXX chez une enfant de* six ans Transmission anormale du groupe Xg *Ann Genet* 11 1.0 1968

Kaufman L Sekhon G S Brazy J E Sivakoff M C & Hatahet G 49XXXXX syndrome in a neonate *Birth Defects* 11 (5) 333 1975

Kesaree L & Wooley P V A phenotypic female with 49 chromosomes presumably XXXXX *J Pediatr* 63 1099 1963

Larger Piet A Bertholot J & Emerit I Une observation du sindrome 49 XXXXX *Nouv Presse Med* 1 467 1972

Lewis F J W Froland A Sanger R & Race R R Source of the X chromosome in two XXXXY males *Lancet* ii 1099 1964

Sergovich F Uilenber C & Pozsonli J The 49 XXXXX chromosome constitution similarity to the 49 XXXXY condition *J Pediatr* 78 285 1971

Yamada Y Nerishi S Penta X (49XXXXX) chromosome constitution a case report *Jap J Hum Genet* 16 15 1971

Submitted May 22 1978
Accepted Febr 19 1979

(P F) I Pediatric Clinic
University of Turin
Piazza Polonia 94
10126 Torino
Italy

Acta Pædiatr Scand 68 773–778 1979

CASE REPORT

NEONATAL RESPIRATORY INSUFFICIENCY DUE TO CENTRONUCLEAR MYOPATHY

B REITTER W MORTIER and L WILLE

From the Children s Hospital University of Heidelberg Heidelberg and the Children s Hospital University of Dusseldorf Dusseldorf FRG

ABSTRACT Reitter B Mortier W and Wille L (Children s Hospital University of Heidelberg Children s Hospital University of Dusseldorf FRG) Neonatal respiratory insufficiency due to centronuclear myopathy Acta Paediatr Scand 68 773 1979 —Two neonates showing generalized hypotonia weakness of limbs trunk and oral musculature died because of muscular respiratory distress The diagnosis of centronuclear (or myotubular) myopathy was established by histological and histochemical techniques The genetic situation and routine laboratory data including electromyography were compared with similar cases in the literature findings were inconclusive with respect to this diagnosis These results indicate the need for a muscle biopsy and the use of histochemical stainings and/or electronmicroscopical investigation for a proper diagnosis in hypotonic newborns under respiratory distress after exclusion of etiologies other than neuromuscular diseases Still the diagnosis of centronuclear myopathy in a neonate does not allow a precise prognosis Increased awareness of this disorder and adequate diagnostic workup is needed in order to extend our understanding and to clarify the prognosis

KEY WORDS Neonates respiratory insufficiency centronuclear myopathy

Severe muscular hypotonia and weakness may cause respiratory insufficiency and in some instances death during the early neonatal period This is known from cases of spinal muscular atrophy (12) and neonatal myasthenia gravis There seems to be an increasing awareness of a similar clinical course in centronuclear or myotubular myopathy one of the congenital structural myopathies which in general allows a good prognosis (17) Since our first case report (18)[1] a second newborn was diagnosed in our hospital and others reported on rather similar clinical and histopathological characteristics (17) In order to help in delineating common features of this malignant variant of centronuclear myopathy we shall detail our two cases

CASE REPORTS

Case 1 A B is the third child of healthy non-consanguineous parents The mother was a 36-year-old gravida III para III Her first pregnancy and delivery were uneventful however the floppy male newborn died at the age of 11 days due to respiratory insufficiency The second pregnancy resulted in a healthy boy who developed

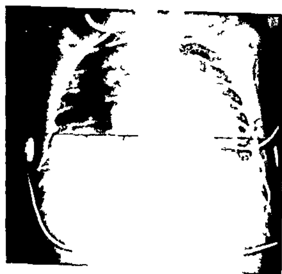


Fig 1 Case 1 roentgenogram of the thorax showing slender ribs and diaphragmatic eventration on the right side

[1] Presented at the meeting of the Deutsche Gesellschaft fur Kinderheilkunde Köln September 1976



Fig 2 Case 1 hypotonic posture unchanged throughout the clinical course

normally During the third pregnancy there were reduced fetal movements

The male infant weighing 2650 g was delivered by caesarean section during the 38th week of gestation Apgar score was six at one minute and seven at five minutes Because of insufficient breathing the newborn required immediate intubation and assisted ventilation On X ray only small ribs and a diaphragmatic eventration on the right side were noted (Fig 1) ECG was according to age During the clinical course constant frog position with extreme generalized muscular hypotonia and sparse spontaneous movements of the limbs and the trunk were noticed (Fig 2) Deep tendon reflexes could hardly be elicited Laboratory data Serum aldolase and creatine kinase (CK) were within normal limits Aldolase 8 1 U/l (=135 nkat/l) CK 83 U/l (=1 383 nkat/l)

An EMG of the anterior tibial muscle and the thenar on the right side corresponded more closely to a neurogenic lesion The motor nerve conduction velocities of the superficial peroneal and the posterior tibial nerve on the right side were normal for his age (29 and 26 m/s respectively)

Two attempts to wean the newborn off the respirator failed and he died on his sixth day

Postmortem examination revealed hypoplasia of the skeletal musculature including the diaphragm On electron microscopy a few myelin sheets of peripheral nerves appeared discontinuous Histologically the striated muscles of different areas including the tongue showed increased variability in fiber size (Fig 3) There was an abnormally high proportion of small fibers About 50% of the muscle fiber nuclei were centrally located and often surrounded by a zone devoid of myofibrils

This was seen more frequently in the fibers with small diameters the intercostal muscles and the tongue showed these myotubular structures to a higher degree than the other muscle groups[1] PAS positive material was increased (Fig 4) Histochemical stainings (NADD-GPDH and ATPase reactions) (Fig 5) confirmed the histological diagnosis of centronuclear myopathy In addition type II fibers outnumbered type I fibers (67 versus 33%) Type II B fibers were almost lacking

---

[1] These postmortem studies were done by PD Dr H P Schmitt and Dr B Volk Neuropathological Institute University of Heidelberg (Director Prof Dr W Ule) The same applies to case 2

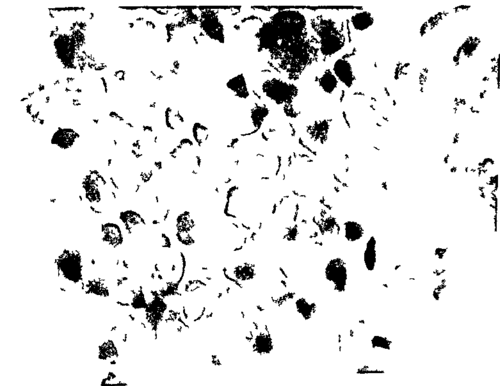


Fig 3 Variation in fiber diameter centrally placed nuclei in the smaller fibers cross section Trichrome ×250

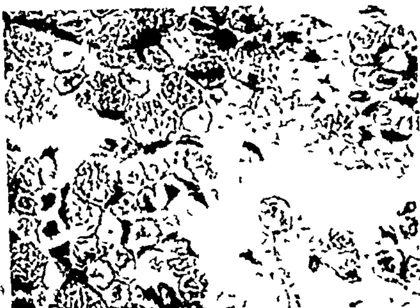


Fig 4 Increased PAS positive material PAS ×250

Case 2 N S first child of healthy not related parents The mother was a 30-year-old gravida I para I During her pregnancy hydramnion and intrauterine dystrophy were suspected Following premature rupture of membranes a female newborn weighing 2770 g was delivered spontaneously at term Apgar notes were two at one minute and three at ten minutes Immediately after birth intubation and ventilatory assistance were necessary because of poor breathing efforts On admission to the newborn intensive care unit chest X rays showed strikingly thin and small ribs and clavicles Clinically there were poor spontaneous movements of the hypotonic limbs decreased tendon reflexes a lack of head control and no fasciculation Transient improvement of spontaneous breathing allowed weaning off the respirator and extubation on the third day However due to progressive shallowness of breathing the newborn had to be reintubed and ventilated again

Laboratory investigation Serum levels of SGOT 60 U/l (=1000 nkat/l) CK 120 U/l (=2000 nkat/l) and aldolase 19 6 U/l (=326 7 nkat/l) were elevated The level of SGPT (36 U/l=600 12 nkat/l) was normal EMG data were obtained from the right rectus femoris and the biceps brachii muscle and had to be limited to a brief

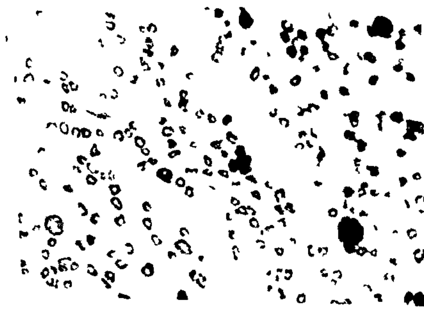


Fig 5 Absence of ATPase activity in central areas of many fibers ATPase preincubated at pH 4 6 ×100



Fig 2 Case 1 hypotonic posture unchanged throughout the clinical course

normally During the third pregnancy there were reduced fetal movements

The male infant weighing 2650 g was delivered by caesarian section during the 38th week of gestation Apgar score was six at one minute and seven at five minutes Because of insufficient breathing the newborn required immediate intubation and assisted ventilation On X ray only small ribs and a diaphragmatic eventration on the right side were noted (Fig 1) ECG was according to age During the clinical course constant frog position with extreme generalized muscular hypotonia and sparse spontaneous movements of the limbs and the trunk were noticed (Fig 2) Deep tendon reflexes could hardly be elicited Laboratory data Serum aldolase and creatine kinase (CK) were within normal limits Aldolase 8 1 U/l (=135 nkat/l) CK 83 U/l (=1393 nkat/l)

An EMG of the anterior tibial muscle and the thenar on the right side corresponded more closely to a neurogenic lesion The motor nerve conduction velocities of the superficial peroneal and the posterior tibial nerve on the right side were normal for his age (29 and 26 m/s respectively)

Two attempts to wean the newborn off the respirator failed and he died on his sixth day

Postmortem examination revealed hypoplasia of the skeletal musculature including the diaphragm On electron microscopy a few myelin sheets of peripheral nerves appeared discontinuous Histologically the striated muscles of different areas including the tongue showed increased variability in fiber size (Fig 3) There was an abnormally high proportion of small fibers About 50% of the muscle fiber nuclei were centrally located and often surrounded by a zone devoid of myofibrils

This was seen more frequently in the fibers with small diameters the intercostal muscles and the tongue showed these myotubular structures to a higher degree than the other muscle groups [1] PAS positive material was increased (Fig 4) Histochemical stainings (NADD-GPDH and ATPase reactions) (Fig 5) confirmed the histological diagnosis of centronuclear myopathy In addition type II fibers outnumbered type I fibers (67 versus 33%) Type IIB fibers were almost lacking

[1] These postmortem studies were done by PD Dr H P Schmitt and Dr B Volk Neuropathological Institute University of Heidelberg (Director Prof Dr W Ule) The same applies to case 2

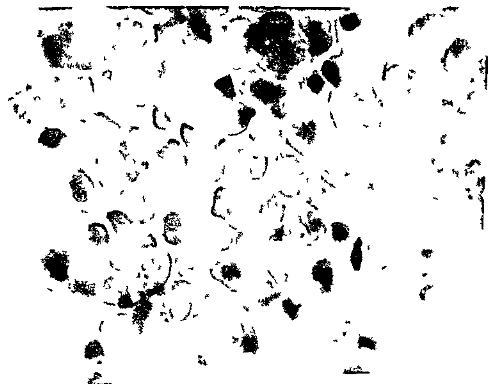


Fig 3 Variation in fiber diameter centrally placed nuclei in the smaller fibers cross section Trichrome

scular disease with pre or perinatal onset ble 1) Reviewing comparable cases in the rature (8 17 22) symptoms include bi ral ptosis facial diplegia and external ilar muscle weakness None of these signs re seen in our two cases In clinical dif ential diagnosis some features of other uromuscular disorders not reported from known cases of neonatal centronuclear opathy might be helpful (Table 2)

Probably all newborns coming to medical at ition because of respiratory distress under X ray examination of the chest The strik g anomaly in both of our children was the nderness of the ribs and clavicles (Fig 1) is phenomenon is known to be associated ith other conditions like myotonic dystrophy) our own experience relates this anomaly to euromuscular diseases with prenatal onset

Determination of muscle enzyme activities ems to be inconclusive for the diagnosis of entronuclear myopathy Raju (17) reported normal CK level one child described here ad a borderline and the other a moderately levated CK activity

Electrophysiological data are not contribu ory (3 6 8 17) Only the histological histo- hemical and electromicroscopical charac ristics of striated muscle can determine the iagnosis of centronuclear myopathy The pecific microscopic findings are described lsewhere in great detail (3 5 7 9 14 16) 21)

However the demonstration of structures sembling myotubes in the patient s muscle iopsy with or without a major degree of fiber pe disproportion still does not provide a pre se prognosis The degree of involvement of fferent muscles and muscle groups appears vary (1) as does the distribution within the oup of muscle fibers (22) One of those uthors judging centronuclear myopathy to be progressive dystrophy primarily limited to e myofibrils (2 3) points to the difference of fibers with central nuclei in a gastro- nemius muscle biopsy compared to 27% in e same muscle at autopsy 17 years later (2)

Therefore quantitative histological results of muscle biopsies will not elucidate the expected course of disease for an individual More pre cision in prognosis is needed for decisions about long term care of affected newborns and for genetic counseling A greater number of diagnosed cases might help to delineate com mon phenomena The short interval elapsing between the cases observed by our group and others allows the assumption that a higher incidence of centronuclear myopathy in neo- nates exists as does an increasing frequency of a proper diagnosis due to awareness of the condition

REFERENCES

1 Barth P G van Wijngaarden G K & Bethlem J X linked myotubular myopathy with fatal neonatal as phyxia *Neurology 25* 531 1975
2 Bradley W G Price D L & Watanabe C K Familial centronuclear myopathy *J Neurol Neuro-surg Psychiatry 33* 687 1970
3 Campbell M J Rebeiz J J & Walton J N Myotubular centronuclear or peri-centronuclear myopathy? *J Neurol Sci 8* 425 1969
4 Chassevent J Sauvegrain J Besson Leaud M & Kalifa G Myotonic dystrophy (Steinert s disease) in the neonate *Radiology 127* 747 1978
5 Coleman R F Thompson L R Niehus A W Munsat T L & Pearson C M Histochemical in-vestigation of myotubular myopathy *Arch Pathol 86* 365 1968
6 Coquet M Vital C Batin J Hehunstre J P & Hedreville M A Un cas de myopathie congénitale associant une hypotrophie des fibres de type I des centralisation nucléaires et une atteinte myofibrillaire focalisee *Arch Anat Cytol Pathol 24* 275 1976
7 Dubowitz V & Brooke M H *Muscle biopsy A modern approach* W B Saunders Company Ltd London 1973 pp 276–279
8 Engel W K Gold G N & Karpati G Type I fiber hypotrophy and central nuclei *Arch Neurol 18* 435 1968
9 Inokuchi T Umezaki H & Santa T A case of type I muscle fiber hypotrophy and internal nuclei *J Neurol Neurosurg Psychiatry 38* 475 1975
10 Karpati G Carpenter S & Nelson R F Type I fibre atrophy and central nuclei A rare familial neuro-muscular disease *J Neurol Sci 10* 489 1970
11 Kinoshita M Satoyoshi E & Matsuo N Myo-tubular myopathy" and type I fiber atrophy" in a family *J Neurol Sci 26* 575 1975
12 Kyllerman M Infantile spinal muscular atrophy (Morbus Werdnig Hoffman) causing neonatal as phyxia *Neuropadiatrie 8* 53 1977
13 McLeod J G Baker De C W Lethlean A K &

Table 1 *Symptoms in centronuclear myopathy causing neonatal respiratory distress*

History of poor fetal movements
Occasional hydramnion
Frog position and lacking head control due to hypotonia
Decreased spontaneous movements due to extreme weakness
Weak cry sucking swallowing
Insufficient breathing
Diminished or absent tendon reflexes

orientation Motor nerve conduction velocities were not measured due to the child s poor condition In the rectus femoris and the biceps muscle shortened potentials and an interference pattern with only slight contraction in the biceps muscle could point to a myopathic process Following aspiration pneumonia developed and caused death on the fourth day of life

Postmortem examination Pale appearance of generally hypotrophic skeletal musculature Microscopy the striated muscle fibers showed increased variability in diameter (5 to 14 μ normal for age 8 to 10 μ) Up to 50% of the nuclei were centrally located They were frequently lacking in the central zones The differentiation of the fiber types remained insecure the ATPase reactions point to a fiber type II predominance

The parents of both children were examined neurologically and electromyographically without pathological findings The mother of the second child underwent muscle biopsy The histological and histochemical results were within normal limits Careful evaluation of the pedigrees of both children did not reveal any factors raising the suspicion of a neuromuscular disorder except with the deceased newborn in the family of case 1

DISCUSSION

Generally the myopathies showing congenital structural anomalies and no histological evidence of inflammation or degeneration are judged to be benign in their clinical course In most cases described hitherto clinical symptoms like muscle weakness and statomotor disablement led to a diagnostic workup beyond infancy However all six patients of the family described by Wijngaarden et al (22) were floppy already in the newborn period and had temporary breathing difficulties While in this family two patients died within the first three weeks of life due to respiratory insufficiency and/resp secondary pulmonary infections, the second family observed by the same authors (1) lost all children affected in the neonatal period In both families a recessive X linked genetic transmission could be traced Barth (1) assumes different alleles on the X chromosome for the explanation of the difference in severity However for clinical decisions even a positive genetic background with clear mode of transmission does not seem very helpful Besides autosomal dominant (10 13 14) and probable autosomal recessive (20) transmission is known for centronuclear myopathy Therefore no common mode of genetic background can be shown so far for the severe clinical involvement Furthermore the coincidence of structural myopathies with in one family (11) as well as within one patient (6 19) has been reported Thus an uneventful family history or the death of another newborn due to centronuclear myopathy does not provide precise information with respect to the individual prognosis of autosomal recessive inheritance or sporadic occurrence

After excluding cardiac and pulmonary etiologies hypotonia with respiratory distress in a newborn should alert the physician to consider a structural myopathy regardless of the family history

The clinical symptoms of a newborn severely affected with centronuclear myopathy hardly differ from those in any other neuro-

Table 2 *Symptoms of neuromuscular disease not reported from centronuclear myopathy in the neonatal period*

Symptom	Disease
Fasciculation	Infantile spinal muscular atrophy
Muscle wasting	Infantile spinal muscular atrophy syndrom of muscle fiber disproportion
Contractures	Congenital muscular dystrophy congenital unspecific myopathy (benign or progressive) arthrogryposis of diff etiology
Myotonic discharges in EMG	Myotonic dystrophy

Acta Pædiatr Scand 68 779-782 1979

CASE REPORT

RENAL TUBULAR ACIDOSIS ASSOCIATED WITH TYPE III GLYCOGENOSIS

J COHEN and M FRIEDMAN

From the Department of Paediatrics Whittington Hospital Highgate Hill London England

ABSTRACT Cohen J and Friedman M (Department of Paediatrics Whittington Hospital London England) Renal tubular acidosis associated with type III glycogenosis Acta Paediatr Scand 68 779 1979 —Two children who presented with severe failure to thrive were found to have Type III glycogen storage disease They both also had defects of tubular acidification an association not previously described The nature of the tubular lesion is characterized and the explanation and therapeutic implications are discussed

KEY WORDS Type III glycogen storage disease renal tubular acidosis

Renal tubular acidosis has been described as a rare association of Type I glycogen storage disease (GSD) (4) but not previously with any of the other types We describe two cases of Type III glycogenosis in each of whom a defect of urinary acidification was found

CASE REPORTS

Case 1

This male infant was born in Doha birth weight 3200 g after an uneventful pregnancy He was well until one year of age when he was noted to be underweight and to have hepatomegaly The father described episodes of pallor and sweating usually occurring before feeds There had been no convulsions His parents were first cousins and in good health There were five siblings all of whom were well

On examination his weight was 6 6 kg 2 2 kg below the third percentile his length was 70 cm ? cm below the third percentile and the head circumference 45 5 cm which lay on the third percentile The only other abnormal finding was a liver edge palpable 3 cm below the costal margin He was developmentally normal Investigations showed haemoglobin 137 g/l urea 3 0 mmol/l sodium 138 mmol/l potassium 4 0 mmol/l bicarbonate 16 mmol/l chloride 10? mmol/l Liver function tests serum and urinary calcium and phosphate were all normal There was no evidence of malabsorption Routine urinalysis was negative Plasma glucose after an overnight fast was 3 6 mmol/l and he had an appropriate response to glucagon but during the course of his admission he had an episode similar to those described by his father during which his plasma glucose was less than 1 mmol/l A liver biopsy performed elsewhere had shown histological features compatible with a diagnosis of GSD but had not been submitted for enzyme histochemistry Subsequent studies on leucocytes and a muscle biopsy showed him to have Type III GSD (Table 1) Further metabolic studies are shown in Table 2 Investigation of renal tubular function revealed an arterial pH of 7 37 bicarbonate 18 mmol/l with a simultaneous urine pH of 7 7 In response to an ammonium chloride load (0 1 g/kg) the urine pH did not fall below 5 38 An overnight water deprivation test was normal as was a urinary aminoacid chromatogram Chest X ray and an intravenous urogram were both normal

Case 2

This thirteen month-old male infant was born in Abu Dhabi at term birth weight 2700 g At the age of three months he was observed to be small a poor feeder and to have delayed motor development Routine urinalysis showed heavy glycosuria in the presence of a normal plasma glucose His parents who were first cousins were well and there were no siblings

On examination his weight was 5 0 kg 3 5 kg below the third percentile his length 60 cm 10 cm below the third percentile and his head circumference 40 cm 4 5 cm below the third percentile The liver edge was palpable 5 cm below the costal margin and his motor development was slightly retarded The remainder of the examination was normal

Initial investigations showed haemoglobin 116 g/l urea 3 0 mmol/l sodium 141 mmol/l potassium 4 3 mmol/l bicarbonate 16 5 mmol/l chloride 110 mmol/l Fasting plasma glucose 3 6 mmol/l Liver function tests showed moderate elevation of serum hydroxybutyrate dehydrogenase to 530 IU/l (0-?90) but were otherwise normal Routine urinalysis showed 2% glucose and was normal on microscopy There was no evidence of malabsorption

Shorey C D Centronuclear myopathy with autosomal dominant inheritance *J Neurol Sci 15* 375 1972
14 Mortier W Michaelis E Becker J & Gerhard I Centronucleäre Myopathie mit autosomal dominantem Erbgang *Hum Genet 27* 199 1975
15 Mortier W Besondere Probleme bei der elektrodiagnostischen Untersuchung von Kindern *Z EEG EMG 8* 36 1977
16 Munsat T L Thompson L R & Coleman R F Centronuclear (myotubular) myopathy *Arch Neurol 20* 120 1969
17 Raju T N K Vidyasagar D Reyes M G & Chokroverty S Centronuclear myopathy in the newborn period causing severe respiratory distress *Pediatrics 59* 29 1977
18 Reitter B Stolzis L Schmitt H P & Scheffner D Perinatale Form der myotubularen Myopathie in der Differentialdiagnose fruhkindlicher neuromuskularer Erkrankungen *Mschr Kinderheilk 125* 498 1977
19 Scelsi R Lanzi G Nespoli L & Poggi P Congenital non progressive myopathy with type II fibre atrophy and internal nuclei *Eur Neurol 14* 285 1976
20 Sher J H Rimalovski A B Athanassiades T J & Aronson S M Familial centronuclear myopathy A clinical and pathological study *Neurology 17* 727 1967
21 Spiro A J Shy G M & Gonatas N K Myotubular myopathy *Arch Neurol 14* 1 1966
22 Van Wijngaarden G K Fleury P Bethlem J & Meyer A E F H Familial myotubular myopathy *Neurology 19* 901 1969

Submitted Sept 15 1978
Accepted Jan 29 1979

(B R) Department of Pediatric Neurology
Univ Kinderklinik
Im Neuenheimer Feld 150
D 6900 Heidelberg
F R G

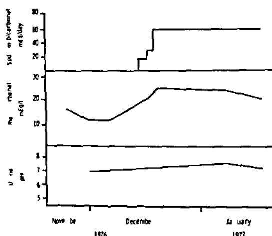


Fig 1 Case 1 Daily measurements of plasma bicarbonate and urinary pH during low dose oral bicarbonate therapy

clinical and biochemical features of Type III glycogenosis which previously have been well described (7) Both presented because of their failure to thrive and in the first case the history was typical of hypoglycaemic attacks Although these classically occur in Type 1 GSD fasting hypoglycaemia is well recognised in the debranching enzyme defect (1) Despite the hepatomegaly mild elevation of the transaminases in the second patient was the only abnormality in liver function tests Increased serum urate and blood lactate are only found in Type I GSD probably as a consequence of prolonged hypoglycaemia The lipoprotein abnormality found in the second case has also been previously described (7)

The definition and classification of renal tubular acidosis (RTA) has been discussed by Sebastian & Morris (6) They have identified three principal types of RTA and various hybrids and it is clear from these descriptions that individual cases may demonstrate considerable overlap Nevertheless it is helpful to try and categorize as far as possible the two patients described here

The first case demonstrated classical distal RTA A mild systemic acidosis was present in the face of a strongly alkaline urine and in response to an ammonium chloride load the urine pH failed to fall below 5 2 on two separate occasions It is difficult to exclude a small degree of bicarbonate wastage from the proximal tubule occurring concurrently and although further studies (e g determination of the tubular maximum for bicarbonate) would help to clarify this they were not thought to be appropriate to the management of this case Subsequent treatment of this patient with low dose sodium bicarbonate (6 mEq/kg/day) effectively reversed the acidosis (Fig 1) and this is consistent with previous experience of treating distal RTA (8)

In the second case there was a more severe hyperchloraemic acidosis in conjunction with an acid urine The additional finding of tubular proteinuria and persistent glycosuria suggested the presence of a partial Fanconi syndrome associated with a proximal bicarbonate wasting RTA

This was tested by giving an oral bicarbonate load Plasma bicarbonate prior to treatment was 10–15 mEq/l Low dose treatment immediately elevated the plasma level to above the threshold for re absorption causing bicarbonate to spill into the urine However it was insufficient to maintain this effect and the plasma bicarbonate rapidly fell to pre treatment levels the urine becoming acid Dou

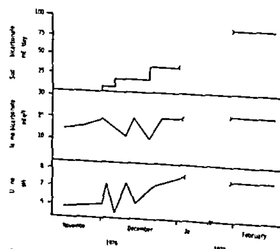


Fig 2 Case 2 Daily measurements of plasma bicarbonate and urinary pH during high dose oral bicarbonate therapy

Table 1 *Summary of enzyme histochemistry confirming both patients to have Type III GSD*

		Patient	Control	Normal range
Case I				
Whole blood glycogen	μg of glycogen ml^{-1} of whole blood	56 6		? 19
Leucocytes	Debranching system μmol glucose min^{-1} mg^{-1} of protein Phosphorylase limit dextrin substrate	0 06		0 6–0 9
Muscle biopsy	Glycogen level	1 4%		<0 ?%
	Debranching activity μmol glucose min^{-1} mg^{-1} of tissue Phosphorylase limit dextrin substrate	0 011	0 66	
	α Schardinger substrate	0 07	0 1?	
Case II				
Leucocytes	Debranching system μmol glucose formed min^{-1} mg^{-1} of protein	0 13		0 6–0 9
Liver biopsy	Glycogen	12%		<4%
	Debranching system μmol glucose min^{-1} mg^{-1} of tissue Phosphorylase limit dextrin substrate	0 05		0 2–0 6
Muscle biopsy	Glycogen	1 3%	0 17%	<0 2%
	Debranching system phosphorylase limit dextrin substrate	0 009	0 66	
	α Schardinger substrate	0 1	0 12	0 8–0 9

and the chest X ray was clear Screening for glycogen storage disease was initially carried out on leucocytes and a diagnosis of Type III GSD later confirmed by percutaneous needle biopsy of the liver and muscle biopsy (Table 1) Subsequent metabolic studies are shown in Table 2

Blood gas analysis showed a systemic acidosis arterial pH 7 34 bicarbonate 12 mmol/l with a simultaneous urinary pH of 5 4 A urinary amino-acid chromatogram was normal and sugar chromatography confirmed glucose only to be present Urinary protien electrophoresis demonstrated beta 2 microglobulin There was no phosphaturia and no defect of tubular concentrating ability

An intravenous urogram was normal To clarify the nature of the renal tubular lesion a bicarbonate loading test was carried out beginning with 2 mEq/kg of 8 4% sodium bicarbonate daily in divided doses by mouth Daily simultaneous measurement of plasma bicarbonate and urine pH were made and the results are shown in Fig 1

DISCUSSION

Type III glycogen storage disease is caused by a relative deficiency of the debranching enzyme system (7) and in both these patients specific enzyme assay revealed this deficiency Both cases demonstrated a number of

Table 2 *Details of further biochemical investigations*

Case II had a mild hypertriglyceridaemia

Investigation	Normal range (mmol/l)	Case I	Case II
Serum uric acid	0 1–0 4	0 36	0 11
Blood lactate	1 0–1 78	1 88	1 33
Fasting serum cholesterol	3 6–6 7	3 4	5 2
Fasting serum triglyceride	0 2–1 6	1 0	2 1
Glucagon test plasma glucose	Fasting	3 6	3 6
	20 min after IM glucagon	6 4	5 0

Acta Pædiatr Scand 68 783–785 1979

CASE REPORT

LOCALIZED SCLERODERMA FOLLOWING VARICELLA IN A THREE YEAR OLD GIRL WITH IgA DEFICIENCY

Z SPIRER B ILIE I A PICK and M YARON

From the Departments of Pediatrics Physical Medicine and Rheumatology Pathology and the Section of Clinical Immunology Rokach (Hadassah) and Ichilov Medical Center Tel Aviv Beilinson Medical Center Petah Tiqva and the Sackler School of Medicine Tel Aviv University Israel

ABSTRACT Spirer Z Ilie B Pick I A and Yaron M (Departments of Pediatrics Physical Medicine and Rheumatology Pathology and the Section of Clinical Immunology Rokach (Hadassah) and Ichilov Medical Center Tel Aviv Beilinson Medical Center Petah Tiqva and the Sackler School of Medicine Tel Aviv University Israel) Localized scleroderma following varicella in a three year-old girl with IgA deficiency Acta Paediatr Scand 68 783 1979.—A three year old girl with isolated IgA deficiency developed localized scleroderma immediately after varicella infection Physiotherapy was started without any drug therapy Only a minimal clinical improvement was achieved The connection between IgA deficiency viral infection and collagen diseases is discussed

KEY WORDS IgA deficiency isolated scleroderma vaircella

Systemic and localized scleroderma are rare diseases in children Chazen et al (4) reported in 1962 nineteen cases of localized scleroderma seen at the Children s Hospital Medical Center in Boston and at the Vanderbilt University Hospital s Pediatric Service between 1935 and 1960 We report here a case of localized scleroderma first noticed after varicella in a girl aged three who also has isolated IgA deficiency

CASE REPORT

A R was born in November 197 after normal gestation and delivery She has a six year-old healthy sister and healthy parents Her birth weight was 3 000 g She was not breast fed and developed normally She was vaccinated against smallpox tetanus pertussis polio and tuberculosis in due course and with no side effects In March 1975 she had mumps She had also suffered from two episodes of otitis media wh h required antibiotics but presented no serious problem In January 1976 she developed varicella with fe er up to 39°C and papular lesions over her entire body Her mother reported that skin lesions were more numerous on her left upper extremity A few days after the onset of varicella her mother noticed that the skin over her entire left upper extremity became tight and the ch ld refrained from using her left arm

Exam nation in February 1976 revealed a pleasant alert and intelligent girl Her height was 95 5 cm and her weight was 13 kg Changes typical for scleroderma were present in the skin of the left upper extremity No changes in the skin of other parts of the body were noticed The skin of the entire left upper extremity was shiny and tightly bound to tissues below There was no dysphagia

Laboratory examinations revealed hemoglobin 126 g/l white count 8 7×10/l with a normal differential sedimentation rate 50/90 (Westergren) total protein 74 g/l albumin 43 g/l globulin 31 g/l Protein paper electrophoresis showed albumin 57% alpha 1 globulin 3% alpha 2 globulin 7% beta globulin 8% and gamma globulin 25% IgG was within the normal range in the patient s serum as well as in the serum of her father mother and sister IgA was absent in the serum and saliva of the patient and of her father but was normal in that of her mother and sister Antinuclear factor was present in the serum anti DNA was 44 9% (maximal normal level in our laboratory is 25%) LE preparation Latex and Rose Waaler tests were negative Total hemolytic complement was 330 units/l (our normal ranges 400–800) C_3 was 56 6 mg/100 ml (our normal ranges 55–120) C was 19 2 mg/100 ml (our normal ranges 20–50) Mantoux test was negative Serum transaminase creatine phosphokinase and aldolase levels were normal Electromyographic examination was normal A biopsy from the left deltoid muscle was performed in February 1976 and showed pathological changes compatible with scleroderma in the skin subcutaneous tissue and adjacent muscle (Fig 1*a* and *b*)

A d agnosis of localized scleroderma was made and physiotherapy was started No drug therapy was given Five months after onset of disease severe scleroderma changes of the entire left upper extremity were even more

bling the dose produced the same effect and eventually a dose of 14 mEq/kg was required to maintain the plasma bicarbonate above the threshold the urine alkaline and to correct the acidosis (Fig 2) The need for this very large dose was consistent with previous experience in treating proximal RTA (8)

In glycogen storage disease absence of the debranching enzyme leads to the accumulation in the liver of abnormal molecules of glycogen with a structure approaching that of the phosphorylase limit dextrin (2) Histological studies of renal biopsy tissue from primary proximal RTA have been essentially normal (10) but in the isolated reports (3 5 9) of the appearances of the renal histology in cases of secondary RTA there is the suggestion that deposition of abnormal metabolites may cause disruption of the tubules in particular

The occurrence together of two such uncommon disorders as Type III GSD and renal tubular acidosis is unlikely to be due to chance alone The children were male and products of a consanguinous marriage and both conditions have an increased familial incidence Indeed enzyme analysis of white cells from the five siblings of the first case demonstrated a brother who had a tenfold reduction in debranching enzyme activity consistent with Type III GSD

The association could thus be explained by genetic linkage alone but equally it is possible to speculate that the accumulation of abnormal molecules of glycogen in the renal tubular cells was responsible for the observed defects of urinary acidification and this concept would be compatible with the findings of a relatively non specific pattern of damage to tubular function

It is important to actively exclude renal tubular lesions as a contributory cause for failure to thrive in all patients with GSD and if demonstrated such defects should be fully characterised since both management and prognosis are materially affected

ACKNOWLEDGEMENTS

We gratefully acknowledge the help of Professor B Ryman and the Department of Biochemistry Charing Cross Hospital London for performing the enzyme analyses

REFERENCES

1 Fernandes J & van de Kamer J H Hexose and protein tolerance tests in children with liver glycogenosis caused by a deficiency of the debranching enzyme system *Paediatrics 41* 935 1968
2 Garancis J C Panares R R Good T A & Kuzma J F Type III Glycogenosis A biochemical and electron microscope study *Lab Invest 22* 468 1970
3 Harris R C & Olav C Liver and kidney glucose 6-phosphatase activity in children with normal and diseased organs *J Clin Invest 46* 336 1954
4 Lampert F Mayer H Tocci P M & Nyhan W L Fanconi syndrome in Glycogen Storage Disease *In* Nyhan (ed) *Amino acid metals and genetic variation* McGraw Hill New York 1967 p 353
5 Morgan H G Stewart W K Lowe K G Stowers J M & Johnstone J H Wilson s disease and the Fanconi syndrome *Quart J Med 31* 361 1962
6 Sebastian A & Morris R C Renal Tubular Acidosis *Clin Nephrol 7* 216 1977
7 Rodney Howell R The Glycogen Storage Diseases *In* J B Stanbury J B Wyngaarden and D S Fredrickson (eds) *The metabolic basis of inherited disease* McGraw Hill New York 1978 4th ed p 137
8 Seldin D W & Wilson J D Renal tubular acidosis *In* J B Stanbury J B Wyngaarden and D S Fredrickson (eds) *The metabolic basis of inherited disease* McGraw Hill New York 1978 4th ed p 1618
9 Schoen E J Lowes Syndrome *Am J Med 27* 781 *1959*
10 Soriano J R Boichis H Stark H & Edelman C M Proximal renal tubular acidosis A defect in bicarbonate reabsorption with normal urinary acidification *Paediatr Res 1* 81 1967

Submitted Nov 14 1978
Accepted March 1 1979

(J C) Renal Unit Department of Medicine
Hammersmith Hospital Royal Postgraduate Medical School Du Cane Rd
London W12 OHS
England

We found no description in the literature of localized scleroderma in a patient with IgA deficiency This finding could be a chance association since selective IgA deficiency could occur without disease status However since IgA deficiency has been found in higher than expected rates in patients with connective tissue diseases (2 3) this might also be the case in localized scleroderma

The onset of the disease following varicella is of interest Viruses may play an important role in autoimmune diseases by adversely modifying the host immune response and promoting the formaton of autoantibodies and autosensitized cells

Moreover in some autoimmune diseases virus particles were identified in affected tissues as shown by Gyorkey et al (5)

We suggest a possible relationship between the development of localized scleroderma in this girl IgA deficiency a virus infection (varicella) and anti DNA antibodies Although the findings mentioned above may be coincidental the development of a severe connective tissue disease in only one limb which was heavily involved by varicella not only bears a serious prognosis for the involved limb but also presents a fascinating enigma which deserves further study

REFERENCES

1 Cassidy J T & Burt A Isolated IgA deficiency in juvenile rheumatoid arthritis *Arthritis Rheum 10* 27? 1967
2 Cassidy J T Friedlander R Schmickel R Petty R & Sullivan D Impaired lymphocyte transformation in patients with connective tissue disease and selective immunoglobulin A (IgA) deficiency *Arthritis Rheum 12* 659 1969
3 Cassidy J T Burt A Petty R & Sullivan D Selective IgA deficiency in connective tissue diseases *N Engl J Med* 280 275 1969
4 Chazen E M Cook C D & Cohen Y Focal scleroderma report of 19 cases in children *J Pediatr 60* 385 196?
5 Gyorkey F Sinkovics J G Min K W & Gyorkey P A morphologic study on the occurrence and distribution of structures resembling viral nucleocapsids in collagen diseases *Am J Med 53* 148 1972
6 Hanson V Drexler E & Kornreich H DNA antibodies in childhood scleroderma *Arthritis Rheum 13* 798 1970

Submitted June 15 1977
Accepted March 14 1979

(M Y) Department of Pediatrics A
Rokach (Hadassah) Hospital
P O Box 51 Tel Aviv
Israel



Fig 1 Thinning of the epidermis flattening of the rete ridges Thickening of the dermis showing hypertrophy and sclerosis of the collagen bundles enclosing groups of sweat glands surrounded by inflammatory infiltrates



Fig 1b The muscle beneath the involved skin shows *interstitial myositis*

prominent than on initial examination they included besides remarkable tightness of the skin vitiligo and shiny skin One year after onset of disease there was no essential change in the clinical findings and atrophy of the left upper extremity was more pronounced Left arm circumference 10 cm above the elbow was 2 5 cm smaller than right arm circumference at the same level Left forearm circumference 6 cm below the elbow was 2 cm smaller than right forearm circumference at the same level Left arm and forearm are each 1 cm shorter than their right counterparts The girl cannot clench her left fist she has no flexion in her left wrist and only 30° wrist extension she lacks 35 of elbow flexion and elbow extension is limited by 35 Abduction of left shoulder is limited by 15 X ray of the left upper extremity showed no calcification

Since her initial visit in February 1976 sedimentation rate gradually decreased to 10/32 (Westergren) anti nuclear factor and anti DNA antibodies disappeared from serum IgA deficiency was reconfirmed

DISCUSSION

In their excellent review of nineteen cases of localized scleroderma in children, Chazen et al (4) stressed the need for further studies in order to elucidate a possible role played by immunological processes in the etiology of this condition Hanson et al (6) found antibodies to DNA in seven out of eighteen children with localized scleroderma while they found none in twenty four children with dermatomyositis During the acute stage of her disease which was accompanied by a high sedimentation rate and diffuse mononuclear infiltration of the involved extremity our patient also had positive antinuclear antibodies and antibodies to DNA but parallel to the return to normal of the sedimentation rate antinuclear antibodies disappeared

Of special interest is the absence of IgA in the girl s as well as in her father s serum and saliva Cassidy et al have described isolated IgA deficiency in juvenile rheumatoid arthritis (1) and other connective tissue diseases (2 3)

NEW BOOKS RECEIVED

D W Smith *Introduction to clinical pediatrics* (2/e) Holt Saunders Ltd Eastbourne £7 00 ISBN 0-7?16-8396-7

G Sterky & L Mellander *Birth weight distribution—an indicator of social development* SAREC Report R 2 1978 95 pp SAREC/WHO Workshop 1978 Copies available on request at SAREC c/o SIDA 105 25 Stockholm Sweden ISSN 0348 ?6 6

R H Pierce M W Mainen & J F Bosma *The cranium of the newborn infant* An atlas of tomography and anatomical sections Superintendent of Documents U S Government Printing Office Washington D C 2040? DHEW Publ No (NIH) 78 788 148 pp $6 50

M E Miller *Host defences in the human neonate* In T K Oliver (Series ed) Monographs in neonatology Grune & Stratton Inc New York San Francisco & London 1978 1?3 pp $1? 50 ISBN 0-8089 1094-9

F Falkner & J M Tanner (eds) *Principles and prenatal growth* vol 1 In Human growth 633 pp illus Plenum Press New York 1978 $4? ISBN 0-306-34461-0

R M Reece Reece Chamberlain *Manuel of emergency pediatrics* (?/e) 7?1 pp Holt Saunders Ltd East bourne 1978 £10 75 ISBN 072167498-4

P Boivin J Boisse H Lestradet & A Gaidos *En ym? pathies* Fascicule V ?3? pp Masson Paris 1978 F 130 - ISBN ?-?5 70158-X

G H Lowrey *Growth and development of children* 7th edition 464 pp Year Book Medical Publishers Inc Chicago London 1978 $ 1 50 ISBN 08151 5644-8

U Stave (ed) *Perinatal physiology* (?nd edition of Physiology of the perinatal period) 851 pp Plenum Publishing Corporation New York 1978 $71 40 ISBN 0306 30999 8

W B Strong (ed) *Atherosclerosis Its pediatric aspects* Grune & Stratton New York 1978 3?9 pp $17 85 ISBN 0-8089 1113 9

L Z Heidam *Fosterskader og kemikalier i a bejds miljøet en litteraturundersøgelse* Odense Universitets forlag 19?8 157 pp Dkr 40 ISBN 87 749? 75-0

E Kerpel Fronius P V Véghelyi & J Rista (eds) *Perinatal medicine* in two parts Akadémiai Kiadó Budapest 1978 1449 pp illustr $1?5 ISBN 963-05 1413 3

R J Touloukian (ed) *Pediatric trauma* John Wiley & Sons New York Chichester Brisbane Toronto 1978 464 pp illus £?4 95 $49 50 ISBN 0-471-01500-8

S E Gerber & G T Mencher (eds) *Early diagnosis of hearing loss* Grune & Stratton New York San Francisco London 1978 377 pp illus $14 50 ISBN 0-8089 1153 8

F Falkner & J M Tanner (eds) *Human growth 2* Postnatal growth Plenum Press New York 1978 634 pp illus $4? 00 ISBN 0-306-344462 9

J Apley *Paediatrics* ?nd ed Concise Medical Text books Baillière Tindall London 1979 453 pp illus £4 95 ISBN 0-70?0-0694-7

E Robertson *Rehabilitation of arm amputees and limb deficient children* 1st ed Baillière Tindall London 1978 ?09 pp illus £8 50 ISBN 0-7020-0714 5

S B Pal (ed) *Enzyme labelled immunoassays of hormones and drugs* Walter de Gruyter Berlin New York 1978 475 pp DM 130 00 ISBN 3 11 007539 3

N R Lundstrom *Echocardiography in congenital heart disease* Elsevier/North Holland Amsterdam New York Oxford 1978 410 pp illus US $66 00 ISBN 0-444 80044 1

R Walter Anyan Jr *Adolescent medicine in primary care* John Wiley & Sons Ltd New York Chichester Brisbane Toronto 1978 387 pp £11 95 ISBN 0-471 03976-4

A Peters *Bewegungsanalysen und Bewegungstherapie im Säuglings und Kleinkinderalter* Gustav Fischer Verlag Stuttgart New York 1977 138 pp illus DM ?6 00 ISBN 3-437-00??8 7

Major mental handicap methods and costs of prevention Ciba Foundation Symposium 59 Elsevier Excerpta Medica/North Holland 1977 236 pp illus No price given ISBN 0-444-90033-0

P G Ransley & R A Risdon *Reflux and renal scarring* British Journal of Radiology Supplement 14 1978 pp illus £3 60 (members of the British Institute of Radiology £3 00) ISBN 0306-8854

A Altman & A Schwartz *Malignant diseases of infancy childhood and adolescence* In A J Schaffer & M Markowitz (eds) Major problems in clinical pediatrics vol XVIII 531 pp illus W B Saunders Company Philadelphia London Toronto 1978 No price given ISBN 0-7?16-1?1? 1

Acta Pædiatr Scand 68 789–794 1979

SOME CHARACTERISTICS OF TRANSCUTANEOUSLY MONITORED OXYGEN PARTIAL PRESSURE IN NORMAL NEWBORNS

O LOFGREN and L JACOBSON

From the Department of Obstetrics and Gynecology General Hospital University of Lund Malmo Sweden

ABSTRACT Lofgren O and Jacobson L (Department of Obstetrics and Gynecology General Hospital University of Lund Malmo Sweden) Some characteristics of transcutaneously monitored oxygen partial pressure in normal newborns Acta Paediatr Scand 68 789 1979 —The transcutaneous oxygen partial pressure (Ptc_O) was monitored in 50 healthy normally and spontaneously delivered newborns Measurements were performed during the first to fourth day of life The electrode temperature was 44 5°C The mean Ptc level recorded during about 45 min was 9 2 kPa (S D 1 4) recorded from the minute to-minute values The Ptc level normally oscillated to a certain extent and the oscillations were closely related to the breathing pattern of the patient When the patient fell asleep during measurement the normal oscillating pattern was replaced by a silent pattern During crying the Ptc level showed four main reaction patterns A decrease in the Ptc level could be observed during breast feeding One child recently fed vomited a small amount of breast milk after a short period of crying and apparently had a laryngospasm shown by a sudden drop in the Ptc level without any other signs of discomfort The study shows that Ptc (and thus also Pa) very sensitively reacts to changes in activity This implies that earlier used methods for determination of Pa might give values that are not representative for the steady state as the sampling method per se might influence the recorded P value

KEY WORDS Transcutaneous monitoring P monitoring normal Ptc_O levels P and state of activity

Transcutaneous oxygen partial pressure (Ptc) is not equivalent to arterial P_O (Pa_O) rather Ptc_O is a new parameter with its own characteristics However Ptc has a very consistent relationship to Pa in each patient and Ptc very reliably reflects even small changes in the central oxygenation given a constant and adequate skin circulation at the measurement site (20)

Systematic studies of the Ptc monitoring method thus far reported have been primarily concerned with the reliability of Pt measured in terms of its correlation with P in intermittently obtained arterial blood samples (5 6 7 9 10 12 14 19 20 24 25 26 27) Most of these studies report on high correlations for *diseased newborns* In some studies however correlations are reported for normal patients breathing air (6 10) and breathing oxygen (10) In 1976 Huch et al (10) reported Ptc_{O_2} to be 8 7 kPa in less than 5% of normal newborns (electrode temperature 45 C and 44 C for normal and preterm infants respectively)

The following study was performed in order to study the range of Ptc_O in normal newborns breathing air during a specified monitoring situation and to study changes in the Ptc_{O_2} level during different states of activity

EQUIPMENT AND PROCEDURE

The equipment used as a Radiometer TCM 1 unit (Radiometer Denmark) A 25 μm teflon membrane was used The electrode temperature was set at 44 5°C (anode temperature the skin surface temperature was assumed to be 43 5°C) Immediately before and after every recording the electrode was calibrated and recalibrated in an

BOOK REVIEWS

A Peters *Bewegungsanalysen und Bewegungstherapie im Säuglings und Kleinkinderalter* Gustav Fischer Verlag Stuttgart New York 1977 138 pp illus DM 26 00 ISBN 3-437 00228 7

The author is physiotherapist in Heidelberg and the book is intended to be of help in the early treatment of infants and pre school children with motor handicaps or late motor development The book is in German It is easy to read and gives a very good description of normal motor development in the first year It also provides several examples of simple ways of treating a child in order to facilitate a normal sensory motor development The figures are very clear and instructive with the child pictured in different positions with arrows marking how the therapist can give support to the child and initiate movement The method of therapy described in the book is greatly influenced by the Bobath method and partly by the Vojta method

This book can be recommended to doctors and therapists dealing with small motor handicapped children and will be especially useful for physiotherapists treating these children

Kerstin Odlund

A Altman & A Schwartz *Malignant diseases of infancy childhood and adolescence In* A J Schaffer & M Markowitz (eds) Major Problems in Clinical Pediatrics vol XVIII 531 pp illus W B Saunders Company Philadelphia London Toronto 1978 No price given ISBN 0-7216-1212 1

It is a difficult task to write on malignant diseases in children when the primary recipient of the book is meant to be the practising pediatrician rather than specialist in the field The authors of the present volume judiciously place much emphasis on diagnosis and the natural history of malignancy as well as on complications of its treatment

The first seven chapters deal with general aspects of cancer in childhood and the chapters on diagnosis and oncologic emergencies are especially valuable The chapter on radiotherapy is concise and informative while that on chemotherapy is overloaded with details The next fifteen chapters describe individual malignancies in a comprehensive and didactic way Some of them such as the chapters on tumours of germ cell origin or on tumours of the sexual organs are doubtless too extensive for the needs of a general pediatrician but may serve as a valuable reference for those specially interested in the field

Figures tables and classification or diagnostic schemes are of high quality throughout the book References are up to date but sometimes unnecessarily numerous

Some minor remarks may be pertinent in case of a 2nd edition of the book

p 200 — description of consolidation therapy in leukemia may be misleading — it does include a short course of chemotherapy to further reduce the leukemic population but not prophylactic treatment of sanctuary areas

p 335 — stage IV S neuroblastoma is described in the text but not exactly defined together with other stages

p 365 — urine should be collected for catecholamine determination in all cases where neuroblastoma is a possible differential diagnosis not only if neuroblastoma is suspected

p 434 — histologic grading of immature teratomas is given both on this page and on p 458 On the other hand no staging system is presented for ovarian neoplasms though treatment and its results are related to stage

On the whole the book can be recommended not only for the general pediatrician for whom it was primarily written but also for the oncologic pediatrician and other members of the pediatric oncology team

Stanislav Garwic

Major mental handicap methods and costs of prevention Ciba Foundation Symposium 59 Elsevier Excerpta Medica/North Holland 1977 236 pp illus No price given ISBN 0-444 90033 0

The book covers a symposium on the cost of preventing major mental handicap held in London November 1977 Most of the participants came from England and hence several of the reported studies related to that country Nevertheless the studies and discussions are relevant to the situation in most western countries

In the symposium major mental handicaps were discussed in relation to prenatal perinatal and postnatal events Several well known investigators from different countries presented their views and own experience of each of these subjects Besides this the possibility of risk assessment during pregnancy and in the perinatal period was discussed Of special interest are the first chapters dealing with the prenatal aspects and stressing the importance of good antenatal care and centralization of delivery and neonatal services The chapters on neonatal intensive care deal mainly with mortality rates but some information on reduced handicap rates is also given

At the end of each presentation there is a discussion that critically analyses what has been presented The last two chapters deal with the application of cost benefit analysis to the prevention of mental handicap both from a theoretical and a practical point of view

This book raises and discusses several important questions Everyone working in the field of perinatology obstetricians and pediatricians should be interested in reading it

Margareta Eriksson

Acta Pædiatr Scand 68 789–794 1979

SOME CHARACTERISTICS OF TRANSCUTANEOUSLY MONITORED OXYGEN PARTIAL PRESSURE IN NORMAL NEWBORNS

O LÖFGREN and L JACOBSON

From the Department of Obstetrics and Gynecology General Hospital University of Lund Malmo Sweden

ABSTRACT Lofgren O and Jacobson L (Department of Obstetrics and Gynecology General Hospital University of Lund Malmo Sweden) Some characteristics of transcutaneously monitored oxygen partial pressure in normal newborns Acta Paediatr Scand 68 789 1979.—The transcutaneous oxygen partial pressure (Ptc_O) was monitored in 50 healthy normally and spontaneously delivered newborns Measurements were performed during the first to fourth day of life The electrode temperature was 44 5°C The mean Ptc level recorded during about 45 min was 9 2 kPa (S D 1 4) recorded from the minute to-minute values The *Ptc* level normally oscillated to a certain extent and the oscillations were closely related to the breathing pattern of the patient When the patient fell asleep during measurement the normal oscillating pattern was replaced by a silent pattern During crying the *Ptc* level showed four main reaction patterns A decrease in the *Ptc* level could be observed during breast feeding One child recently fed vomited a small amount of breast milk after a short period of crying and apparently had a laryngospasm shown by a sudden drop in the *Ptc* level without any other signs of discomfort The study shows that *Ptc* (and thus also Pa_O) very sensitively reacts to changes in activity This implies that earlier used methods for determination of *Pa* might give values that are not representative for the steady state as the sampling method per se might influence the recorded *P* value

KEY WORDS Transcutaneous monitoring *P* monitoring normal *Ptc* levels *P* and state of activity

ranscutaneous oxygen partial pressure ʼtc) is not equivalent to arterial P_O ʼa,) rather Ptc_{O_2} is a new parameter ith its own characteristics However tc, has a very consistent relationship) Pa, in each patient and Ptc_{O_2} very :liably reflects even small changes in the entral oxygenation given a constant and dequate skin circulation at the measurement ite (20)

Systematic studies of the Ptc_O monitor ng method thus far reported have been pri narily concerned with the reliability of ʼtc, measured in terms of its correla ion with P, in intermittently obtained arter ial blood samples (5 6 7 9 10 12 14 19 20 24 25 26 27) Most of these studies report on high correlations for *diseased newborns* In some studies however correlations are reported for normal patients breathing air (6 10) and breathing oxygen (10) In 1976 Huch et al (10) reported Ptc_{O_2} to be 8 7 kPa in less than 5% of normal newborns (electrode temperature 45 C and 44 C for normal and preterm infants respectively)

The following study was performed in order to study the range of Ptc_O in normal newborns breathing air during a specified monitoring situation and to study changes in the Ptc_{O_2} level during different states of activity

EQUIPMENT AND PROCEDURE

The equipment used as a Radiometer TCM 1 unit (Radiometer Denmark) A 25 μm teflon membrane was used The electrode temperature was set at 44 5 C (anode temperature the skin surface temperature was assumed to be 43 5 C) Immediately before and after every recording the electrode was calibrated and recalibrated in an

Table 1 Ptc_{O_2} in normal newborns

N	Electrode temp (C)	Electrode drift (kPa)	Mean Ptc_{O_2} (kPa)	Mean birth weight (g)	Mean recording time (min)	Mean measuring time (min)
50	44 5	−0 2 (S D 0 8)	9 2 (S D 1 4)	3 579 (S D 448 0)	44 5 (S D 11 70)	60 0 (S D 6 11)

antiseptic water solution of Urolicide* (American Latex Corporation Indiana USA) bubbled through with air The temperature of the calibration solution was 43 5 C

The infants usually recently fed and changed were supine on a nursing table and partly covered by blankets The room temperature was approximately 25 C A newly calibrated electrode was applied by means of a double adhesive tape in the subclavicular area After stabilization of the Ptc_{O_2} level recording was performed for about 45 min During the recording time the spontaneous fluctuations of the Ptc_{O_2} levels were studied as was the average basal level At the end of the recording some of the infants were made to cry by tapping the sole of the foot In two cases the infant was given to the mother for breast feeding at the end of the recording with the electrode still in place After the recording the electrode was recalibrated

PATIENTS

Recordings were performed in 50 healthy normally and spontaneously delivered newborns with a mean birth weight of 3 579 g (Table 1) The measurements were performed during the first to fourth day of life (Fig 1)

RESULTS

Electrode drift

The mean electrode drift obtained as the difference in calibration values before and after the recording was −0 2 kPa (S D 0 8) showing a small mean declining drift (Table 1)

Ptc_{O_2}

All recordings showed a more or less wave like pattern It was apparent that although the pattern was dependent on the activity of the infant it could also differ among infants in the same state of agitation Thus when the infant was alert or irritated the oscillations were frequent with comparatively high amplitude However when the newborn was asleep smooth pattern (silent pattern) appeared An example of this is shown in Fig 2 with the infant alert but not crying during the first part of the recording and asleep during the latter part

The maximal range within which the Ptc_{O_2} level oscillated in the same patient was 6 4 kPa and the minimal range of oscillations recorded in one patient was 0 6 kPa The range of Ptc_{O_2} most commonly recorded among patients was about 2 7 kPa

Ptc_{O_2} was calculated minute to minute and from these values a mean Ptc_{O_2} level was obtained for the patients The mean Ptc_{O_2} level was obtained for the patients The mean Ptc_{O_2} level recorded was for the total material 9 2 kPa (S D 1 4) (Table 1)

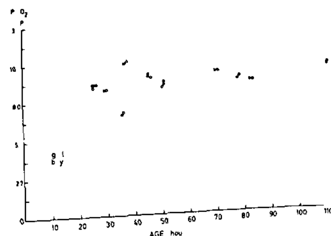


Fig 1 Material Age at start of measurement and mean Ptc_{O_2} level calculated from the minute to minute values

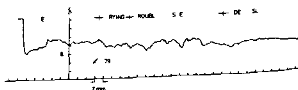


Fig 2 The effect of different stages of activity on Ptc_{O_2} level

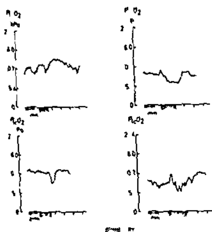


Fig 3 Four common reaction patterns of the $P_{tc_{O_2}}$ level during crying

Crying was produced in 30 of the 50 infants by tapping the sole of the foot for about 2 min. During the crying period the Ptc_{O_2} level showed varying reaction patterns. The four most common patterns are shown in Fig 3. In most infants after an initial brief increase the Ptc_{O_2} level started to decrease (Fig 3 1 2 4). The mean decrease in the Ptc_{O_2} level during crying was 2 2 kPa (S D 1 0). Eleven of the infants became calm again immediately after two min crying, the Ptc_{O_2} levels in these children returned to their original levels on the average 75 sec after crying had stopped (Fig 3 2). In five of these 11 infants the Ptc_{O_2} level continued to fall even though the child was calm. In these latter five infants the Ptc_{O_2} level reached a mean maximal decrease of 3 4 kPa on the average 34 sec after they had stopped crying (Fig 3 3). The original Ptc_{O_2} level was reached after a mean of 104 sec. In two children the Ptc_{O_2} level increased immediately when they started to cry as in most cases but then fell only a few kPa below the original level during the 2 min long crying period (Fig 3 1).

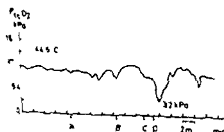


Fig 4 Reaction of the Ptc_{O_2} level to a brief laryngospasm after vomiting (D). (A) Troubled sleep, (B) crying, (C) stop of crying, (D) vomiting.

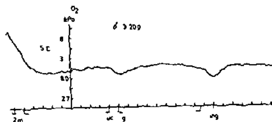


Fig 5 Reaction of the Ptc_{O_2} level to breast feeding

During one recording shown in Fig 4 the recently fed infant vomited a small amount of breast milk after a short crying period. After this the child apparently had a laryngospasm shown by a sudden drop in the Ptc_{O_2} level recorded. The Ptc_{O_2} level decreased to a minimal level of 3 3 kPa. The child was lying on its back almost asleep during the incident. No cyanosis or obvious signs of discomfort could be observed by the experimenter. The child was observed intensively without any intervention by the experimenter; after a few sec the Ptc_{O_2} level increased to its original and normal level and remained there for the rest of the recording. The subsequent course was uneventful.

At the end of the recordings two infants were breast fed by their mothers with the electrodes still in place (Fig 5). A decrease in the Ptc_{O_2} level could be observed during sucking in these newborns as in those given a dummy teat for consolation when crying during the first part of the recording. When the baby intermittently stopped sucking the Ptc_{O_2} level immediately rose to its original level.

DISCUSSION

Ptc_{O_2} should be regarded as a new parameter with its own characteristics such as

normal range and normal value A normal range is by definition the range between two extreme values observed in a defined population by a well defined laboratory procedure For Ptc_{O_2} measurements the laboratory procedure is not well defined and some studies do not even report on the laboratory procedure used

Fenner et al (6) and Huch et al (10) have reported mean Ptc_{O_2} levels of 12 0 kPa and 11 5 kPa respectively in newborns In both studies Ptc_{O_2} was reported as intermittent values related to P_{O_2} in arterial samples obtained by puncture of the radial artery The skin temperature was 43 0°C in both studies

Huch et al (13) reported a normal range of arterial P_{O_2} in non crying neonates from about 8 7 kPa to 13 4 kPa In only 5% the Ptc_{O_2} level was said to be less than 8 7 kPa in non crying neonates The electrode temperature used for fullterm infants was 45°C and for premature infants 44°C The equipment used was the original Huch instrument with three cathodes

The discrepancy between the mean Ptc_{O_2} level obtained in the current study and the mean level reported by others could to some extent be accounted for by differences in the calibration procedure and differences in electrode temperature It must be stressed as mandatory to report on both the calibration temperature and on the electrode temperature used during measurement in reports of Ptc_{O_2} For the current instrument the electrode temperature 44 5 C has been shown to produce a high correlation between Pa_{O_2} and Ptc_{O_2} (20) and in measurements with two simultaneously operating electrodes the electrode temperature (anode temperature) of 44 5 C has been shown to produce an optimal arterialization (21)

The mean Ptc_{O_2} level obtained in the current study concurs with the level reported for Pa_{O_2} obtained intermittently from umbilical artery catheters in healthy newborns (16 17)

As was shown in the current study crying usually started with a short period of tachypnea and hyperventilation This produced an initial increase of the Ptc_{O_2} level followed by a decrease Thus puncture of the radial artery per se affects both the Pa_{O_2} and the Ptc_{O_2} level so that the P_{O_2} recorded by both methods would not reliably reflect P_{O_2} in the steady state Other studies with the current instrument and the current laboratory procedure (19 20) have shown a high correlation between Ptc_{O_2} and arterial samples obtained from umbilical artery catheters with only a very small difference between Pa_{O_2} and Ptc_{O_2} during normoxemia

The present study showed that the pattern of oscillation was dependent on the breathing pattern of the infant and thus on the state of activity When the child was alert or irritated the Ptc_{O_2} level showed vigorous oscillations These oscillations were smoothed out and changed for a silent pattern if the child was asleep The mean level of Ptc_{O_2} seemed to be the same during both states of activity however intermittent apnea i e during breast feeding and sucking could result in rather deep and broad dips of the Ptc_{O_2} level Crying almost always resulted in a decrease of the Ptc_{O_2} level after 5–10 sec This has also been found by others (8 10 11 14) However the Ptc_{O_2} response to crying does not seem to be uniform showing four main patterns in the present study Usually the Ptc_{O_2} level decreased during crying and returned to a normal level when the child stopped crying In some infants the decrease of the Ptc_{O_2} level continued after the crying had stopped Previous studies of the physiology of crying patterns in children have mostly focused on respiratory volumes (18) The Ptc_{O_2} monitoring method thus offers a new possibility for studying the physiology of crying as certain crying techniques increase the Ptc_{O_2} level whereas others cause a decrease in the Ptc_{O_2} level

During recording one infant had a laryngospasm due to vomiting The immediate decrease in the recorded Ptc_{O_2} level is evidence of the sensitivity of the method The child s Ptc_{O_2} level declined to a very low level for a

short time although there was no sign of discomfort The condition was spontaneously relieved Incidents of this kind are probably very common in newborns If however the P_{O_2} level of the infant were initially low the brief apnea of this type could prove harmful beginning a vicious circle (2 3 for a review see 15) A laryngeal chemosensitivity producing apnea has even been proposed as one of several possible mechanisms accounting for sudden infant death (1 4)

The electrode drift calculated is the difference in calibration value before and after the recording was low Lofgren (22 23) has shown that the electrode drift calculated in this way is unsatisfactory as a standard for calculation of the electrode drift during measurement That finding also corresponds to ideas suggested by James (14) The Ptc_{O_2} oscillatory pattern—and perhaps also the stabilization pattern—seem to be better indices of the reliability of the Ptc_{O_2} *recording than is* the recalibration value (19) It has been suggested (19) that a silent pattern found in a spontaneously breathing patient who is *not* sleeping might indicate that the *electrode is* applied in a non optimal measurement site giving a less reliable recording

REFERENCES

1 Barker T L & McGinty D J Reversal of cardiopulmonary failure during active sleep in hypoxic kitten Implications for sudden infant death *Science* 198 419 1977

Brady J P & Ceruti E Chemoreceptor reflexes in the newborn infant Effects of varying degrees of hypoxia on heart rate and ventilation in a warm environment *J Physiol* 184 631 1966

3 Cross K W & Oppé T E The effect of inhalation of high and low *concentrations* of oxygen on the respiration of the premature infant *J Physiol* 117 38 195

4 Downing S E & Lee J C Laryngeal chemosensitivity *A possible* mechanism for sudden infant death *Pediatrics* 55 640 1975

5 Eberhard P Mindt W Jann F & Hammacher K Oxygen monitoring of newborns by *skin electrodes* Correlation between arterial and cutaneously determined PO *In* F Bruley & R E Bicher (eds) *Oxygen Transport to Tissue* Plenum Press New York 1973

6 Fenner A Muller R Busse H G Junge M & Wolfsdorf J Transcutaneous determination of arterial oxygen tension *Pediatrics* 55 224 1975

7 Friis Hansen B Marstrand Christiansen P & Vesterager P Transcutaneous measurement of arterial blood oxygen tension with a new electrode *In* L Stern B Friis Hansen & P Kildeberg (eds) *Intensive Care in the Newborn* Masson Publishing Inc New York 1976 p 259

8 Haschke F Hackel F & Hohenauer L Erfahrungen mit der transkutanen (unblutigen) PO Registrierung beim Atemgestorten Neugeborenen *Klin Paediatr* 188 73 1976

9 Huch A Huch R & Lubbers D W Quantitative polarographische Sauerstoffdruckmessung auf der Kopfhaut des Neugenorenen *Arch Gynaekol* 207 443 1969

10 Huch R Huch A Meinzer K & Lubbers D W *Eine beheizte Pt oberflachen elektrode ur kontinuierlichen Uberwachung des PO beim Menschen* Vortrag Medizin Technik Stuttgart 1972

11 Huch R Huch A & Lubbers D W Transcutaneous measurement of blood PO ($tcpO_2$)—Method and application in perinatal medicine *J Perinat Med* 1 183 1973

12 Huch R Lubbers D W & Huch A Reliability of transcutaneous monitoring of arterial PO_2 in newborn infants *Arch Dis Child* 49 213 1974

13 *Huch R* Huch A & Lubbers D W Die kontinuierliche Kontrolle des *arteriellen* PO_2 und ihre Bedeutung für die Uberwachung von Mutter und Kind *Biomed Tech* 19 87 1974

14 James L S Development and clinical evaluation of blood *gas sensors* for continuous monitoring (adult and neonate) *US Dept of Commerce National Technical Information Service* 1 1976

15 Kattwinkel J Neonatal apnea Pathogenesis and therapy *J Pediatr* 90 342 1977

16 Koch G & Wendel H Comparison of pH carbon dioxide tension standard bicarbonate and *oxygen* tension in capillary blood and in arterial blood during the neonatal *period Acta Paediatr Scand* 56 10 1967

17 Koch G & Wendel H Adjustment of arterial blood gases and acid base *balance* in the normal newborn infant during the first week of life *Biol Neonat* 12 136 1968

18 Long E C & Hull W E Respiratory volume flow *in* the crying newborn infant *Pediatrics* 7 373 1961

19 Löfgren O *On transcutaneous PO₂ measurements in humans Some methodological physiological and clinical studies* Thesis University of Lund 1978

20 Löfgren O Henriksson P Jacobson L & Johansson Ö Transcutaneous PO_2 *monitoring* in neonatal intensive care *Acta Paed atr Scand* 67 693 1978

21 Lofgren O & Jacobson L The influence of different electrode temperatures on the recorded transcutaneous PO_2 level Accepted for publication *Pediatrics*

Löfgren O The recalibration value after PO measurement Submitted for publication

normal range and normal value. A normal range is, by definition, the range between two extreme values observed in a defined population by a well defined laboratory procedure. For Ptc_{O_2} measurements the laboratory procedure is not well defined and some studies do not even report on the laboratory procedure used.

Fenner et al. (6) and Huch et al. (10) have reported mean Ptc_{O_2} levels of 12.0 kPa and 11.5 kPa respectively in newborns. In both studies Ptc_{O_2} was reported as intermittent values related to P_{O_2} in arterial samples obtained by puncture of the radial artery. The skin temperature was 43.0 C in both studies.

Huch et al. (13) reported a normal range of arterial P_{O_2} in non-crying neonates from about 8.7 kPa to 13.4 kPa. In only 5% the Ptc_{O_2} level was said to be less than 8.7 kPa in non-crying neonates. The electrode temperature used for fullterm infants was 45 C and for premature infants 44°C. The equipment used was the original Huch instrument with three cathodes.

The discrepancy between the mean Ptc_{O_2} level obtained in the current study and the mean level reported by others could to some extent be accounted for by differences in the calibration procedure and differences in electrode temperature. It must be stressed as mandatory to report on both the calibration temperature and on the electrode temperature used during measurement in reports of Ptc_{O_2}. For the current instrument the electrode temperature 44.5 C has been shown to produce a high correlation between Pa_{O_2} and Ptc_{O_2} (20) and in measurements with two simultaneously operating electrodes the electrode temperature (anode temperature) of 44.5°C has been shown to produce an optimal arterialization (21).

The mean Ptc_{O_2} level obtained in the current study concurs with the level reported for Pa_{O_2} obtained intermittently from umbilical artery catheters in healthy newborns (16, 17).

As was shown in the current study, crying usually started with a short period of tachypnea and hyperventilation. This produced an initial increase of the Ptc_{O_2} level followed by a decrease. Thus puncture of the radial artery per se affects both the Pa_{O_2} and the Ptc_{O_2} level, so that the P_{O_2} recorded by both methods would not reliably reflect P_{O_2} in the steady state. Other studies with the current instrument and the current laboratory procedure (19, 20) have shown a high correlation between Ptc_{O_2} and arterial samples obtained from umbilical artery catheters with only a very small difference between Pa_{O_2} and Ptc_{O_2} during normoxemia.

The present study showed that the pattern of oscillation was dependent on the breathing pattern of the infant and thus on the state of activity. When the child was alert or irritated the Ptc_{O_2} level showed vigorous oscillations. These oscillations were smoothed out and changed for a "silent pattern" if the child was asleep. The mean level of Ptc_{O_2} seemed to be the same during both states of activity, however intermittent apnea, i.e. during breast feeding and sucking, could result in rather deep and broad "dips" of the Ptc_{O_2} level. Crying almost always resulted in a decrease of the Ptc_{O_2} level after 5–10 sec. This has also been found by others (8, 10, 11, 14). However, the Ptc_{O_2} response to crying does not seem to be uniform, showing four main patterns in the present study. Usually the Ptc_{O_2} level decreased during crying and returned to a normal level when the child stopped crying. In some infants the decrease of the Ptc_{O_2} level continued after the crying had stopped. Previous studies of the physiology of crying patterns in children have mostly focused on respiratory volumes (18). The Ptc_{O_2} monitoring method thus offers a new possibility for studying the physiology of crying, as certain crying techniques increase the Ptc_{O_2} level whereas others cause a decrease in the Ptc_{O_2} level.

During recording one infant had a laryngospasm due to vomiting. The immediate decrease in the recorded Ptc_{O_2} level is evidence of the sensitivity of the method. The child's Ptc_{O_2} level declined to a very low level for a

Acta Pædiatr Scand 68 795–801 1979

TRANSEPIDERMAL WATER LOSS IN NEWBORN INFANTS

III Relation to Gestational Age

K HAMMARLUND and G SEDIN

From the Department of Paediatrics University Hospital Uppsala Sweden

ABSTRACT Hammarlund K and Sedin G (Department of Paediatrics University Hospital Uppsala Sweden) Transepidermal water loss in newborn infants III Relation to gestational age Acta Paediatr Scand 68 795 1979 —Using a method described earlier the evaporation rate (ER) was studied at different humidities in 12 newborn infants born after 25 to 30 weeks of gestation and 10 infants born after 32 to 35 weeks Transepidermal water loss (TEWL) was estimated in 32 infants born after 25 to 39 weeks of gestation The ER values were highest in the infants with the lowest gestational age and the susceptibility to changes in ambient humidity was also greater at lower gestational ages An exponential relationship was found between TEWL and gestational age TEWL being 15 times higher in infants born after 25 weeks of gestation than in full term infants

KEY WORDS Water loss water balance newborn infants neonatal intensive care gestational age

Water electrolytes and calories are frequently supplied by the parenteral route in newborn infants when oral feeding is difficult for some reason e g prematurity asphyxia or pulmonary disease The amount of fluid to be given is usually calculated in relation to body weight or body surface area with adjustments according to postnatal age

Disturbances of water balance in the neonatal period are not due solely to the supply of fluid to the infant Other factors to be considered are water losses to the environment and changes in the body distribution of water (4)

Hitherto no accurate clinical and laboratory methods for routine checking of the state of hydration and of insensible water loss (IWL) have been available IWL the major part of which is cutaneous (9) has mainly been investigated in full term infants or infants with a low birth weight while no thorough study of this loss has been made in relation to gestational age and environmental factors

In our earlier investigations of the evaporation rate (ER) from the skin of full term infants a clear relationship between ER and ambient humidity was found and a method for determination of transepidermal water loss (TEWL) was proposed (5 15) Changes in TEWL in relation to activity and body temperature have been found (6 7 16) A very high TEWL in preterm infants has been noted and reported in preliminary communications (7 16)

The purpose of this investigation was to study the evaporation rate from the skin of newborn preterm infants during the first day of life and to estimate the TEWL in relation to gestational age

SUBJECTS

ER was measured at different ambient humidities on 12 infants born after 25 to 30 weeks of gestation and on 10 infants born after 32 to 35 weeks of gestation (Table 1 measurement series 1*a* and 1*b*) Further measurements of ER were performed and TEWL was estimated (5) in 32 infants born after 25 to 39 weeks of gestation (Table 1 measurement series 2) All infants were appropriate for gestational age (AGA 10) The gestational age was estimated from pregnancy data and as described by Finnström (3) and also in most cases with the cri

23 Lofgren O Drift of the transcutaneous oxygen monitoring electrode during in vivo measurement Submitted for publication
24 Swanstrom S Villa Elisiga I Cardona L Cardenes A Méndez Bauer C & Rooth G Transcutaneous PO_2 measurements in seriously ill newborn infants *Arch Dis Child 50* 913 1975
25 Swanstrom S & Rooth G The value of transcutaneous PO_2 measurements in a newborn infant in shock *Paediatrician 5* 304 1976
26 Versmold H Hannes V Hopner F & Riegel K P Kritische Anmerkungen zur transkutanen PO_2 Messungen bei Risikoneugeborenen *Monatsschr Kinderheilkd 123* 459 1975
27 Versmold H Onken D Hopner F & Riegel K Transcutaneous monitoring of PO in the sick newborn *In* L Stern B Friis Hansen & P Kildeberg (eds) *Intensive Care in the Newborn* Masson Publishing USA 1976 p 269

Submitted March 22 1979
Accepted May 8 1979

(O L) Department of Obstetrics and Gynecology
Allmanna Sjukhuset
S 214 01 Malmo
Sweden

Table 2 *Skin temperature (T_{skin}) and ambient temperature (T_{amb}) in series 1 and 2*

	Series 1		Series 2	
Infant	T_{skin} ± S D (°C)	T_{amb} ± S D (°C)	$\bar{T}_{skin}$ (°C)	$\bar{T}_{amb}$ (°C)
1	36 2±0 1	39 3±0 5	36 1	38 3
			36 6	38 8
3	35 9±0 1	39 8±0 7	35 9	39 9
4	36 0±0 2	39 6±0 7	36 0	39 0
5	36 4±0 1	38 6±0 9	36 5	39 0
6	36 5±0 1	38 8±0 5	36 3	38 9
7	36 6±0	39 5±0 4	36 5	39 8
8	36 4±0 3	36 5±0 6	36 6	36 9
9	35 7±0 3	36 7±0 7	35 8	36 4
10	36 2±0 3	37 3±1 0	36 5	36 6
11	36 0±0 1	35 3±0 8	36 4	34 0
12	36 3±0 0	37 5±0 4	36 5	37 8
13	36 0±0 1	36 6±0 6	35 8	36 5
14	36 5±0 0	36 1±0 5	36 1	35 5
15	36 1±0 1	35 3±0 5	36 1	32 6
16	36 3±0 1	34 6±0 2	36 1	34 6
17	35 6±0	34 8±0 4		
18	36 1±0 1	34 4±0 6	36 0	34 0
19	35 5±0 0	33 7±0 7	35 3	33 4
0	34 9±0 3	34 2±0 9		
1	35 9±0 1	34 5±0 4	36 1	34 7
	35 9±0 1	34 8±0 4	35 9	34 9
3			36 2	33 8
4	35 6±0 1	35 7±0 7	35 8	35 1
5			35 1	33 4
6			35 5	35 0
7			36 2	34 0
8			36 3	35 8
9			35 7	33 2
30			35 6	33 5
31			35 4	33 8
3			35 0	32 8
33			34 8	33 2
34			36 0	34 4

it was possible to keep T_{skin} fairly constant In all but three of the infants with a gestational age of less than 35 completed weeks the system for servo-control of skin temperature (AGA MK138 AGA Medical Lidingö Sweden) supplied with the incubator was used

To keep T_{skin} between 36 0 and 37 0°C the servo-control was set at approximately 36 5°C and the regulating thermistor was attached to the skin of the abdomen The servocontrol adjusted the temperature in the incubator to a level high enough to maintain the abdominal skin temperature at the set level As a safety circuit interrupts the supply of heat when the temperature of air circulating in the incubator reaches about 40°C some infants with a low gestational age did not reach an abdominal skin temperature of 36 5 C The highest T_{amb} was thus registered for infants of the shortest gestational ages (Tables 1 and) both in series 1 and in series

When the servocontrol was not used (infants of more than 34 completed weeks of gestation) the temperature in the incubator was set at 33 0–35 5°C depending on the temperature used in the period before the measurements

1 *Measurement of ER at different ambient humidities*

With the infant in the prone position intermittent measurements of ER were made on an interscapular skin surface while RH_{amb} was increased in steps from 20% to 60% or decreased from 60% to 20% through a change in the relation between dried and humidified air in the incubator at a constant air flow After each change in RH_{amb} steady state was reached in 5–10 min

Estimation of transepidermal water loss

With the infant in the lateral position and with RH_{amb} maintained at 50% ER was measured on the chest (*a*) on an interscapular skin area (*b*) and on a buttock (*c*) The transepidermal water loss (TEWL g/m²h) i e the cutaneous water loss per unit area was calculated as described previously (5) using the equation

$$\mathrm{TEWL}=0\ 9\ \overline{\mathrm{ER}}_{(a,\ b,\ c)}+1\ 37 \qquad (1)$$

Skin temperatures were recorded from the areas where the ER measurements were made

TREATMENT OF DATA

Data were obtained for ER RH_{amb} $P_{H_2O\ amb}$ T_{skin} T_{amb} and T_{body}

The arithmetic mean of the three measured skin temperatures for each TEWL was designated $\bar{T}_{skin}$ (Table 2) Regression analysis was performed by a computer

RESULTS

1*a* *Evaporation rate at different ambient humidities in infants born after 32 to 35 weeks of gestation compared with that in full term infants*

When ER was measured on an interscapular skin area in the 10 infants born after 32 to 35 weeks of gestation higher values were obtained at a low RH_{amb} than at a high one (Fig 1 upper curve) This is consistent with the findings in fullterm infants (Fig 1 lower curve) made in a previous study (5) The values obtained in these preterm infants were higher than those in full term infants at corresponding RH_{amb} For example at an RH_{amb} of 50% ER was 6 2 g/m²h compared with 3 5 g/m²h in the full term infants

Table 1 *Infant data in series 1 and 2*

w = completed weeks of gestation V = vaginal delivery CS = Caesarean section

Infant	Gestational age (w)	Delivery	Weight at birth (kg)	Length at birth (m)	Sex	Measurement series
1	25	V	0.825	0.350	F	1b 2
2	25	V	0.830	0.330	F	2
3	26	V	0.950	0.350	M	1b 2
4	26	CS	0.680	0.330	M	1b 2
5	27	V	1.070	0.360	M	1b 2
6	28	V	1.070	0.355	M	1b 2
7	28	CS	0.890	0.350	M	1b 2
8	29	V	1.600	0.410	F	1b 2
9	29	V	1.570	0.410	M	1b 2
10	30	CS	1.370	0.400	F	1b 2
11	30	V	1.420	0.405	F	1b 2
12	30	V	1.500	0.400	M	1b 2
13	30	CS	1.330	0.360	M	1b 2
14	32	CS	1.590	0.435	M	1a 1b 2
15	32	CS	1.670	0.405	M	1a 1b 2
16	33	V	2.150	0.430	F	1a 1b 2
17	33	CS	2.0[illegible]0	0.430	F	1a 1b
18	33	CS	2.090	0.450	M	1a 1b 2
19	33	V	1.900	0.440	M	1a 1b 2
20	34	CS	2.160	0.450	F	1a 1b
21	34	CS	2.280	0.460	M	1a 1f 2
22	34	V	2.000	0.450	F	1a 1b 2
23	34	V	2.130	0.455	M	2
24	35	V	2.320	0.450	M	1a 1b 2
25	38	V	3.070	0.470	F	2
26	38	CS	3.730	0.570	M	2
27	38	CS	3.620	0.525	M	2
28	38	CS	3.990	0.510	M	2
29	38	CS	3.750	0.500	M	2
30	38	CS	2.910	0.490	M	2
31	38	CS	3.770	0.500	M	2
32	39	V	3.680	0.530	M	2
33	39	V	2.850	0.500	F	2
34	39	CS	2.960	0.485	F	2

term proposed by Dubowitz et al (1) The ER measurements were carried out during the first 24 h after delivery The mean age at the start of the measurements was 10 7 h in series 1 and 8 1 h in series 2 The duration of the measurements was 1 8 h in series 1 and about 10 min in series 2 The infants were often asleep when the measurements were being made When awake they were quiet and calm and showed little spontaneous motor activity (cf 6) The respiratory and heart rates were normal for age

METHODS

ER was measured by a method based on determination of the vapour pressure gradient in the air layer close to the skin surface (12 13 14) This method which has been used earlier in studies on water loss in term infants (5 6) allows accurate measurements and does not interfere with nursing routines The equipment for measurement of ER also gives data on the ambient relative humidity (RH_{amb}) and ambient vapour pressure ($P_{H_2O\,amb}$) The ambient air temperature (T_{amb}) skin temperature (T_{skin}) and body temperature (T_{body}) were measured with a YSI telethermometer (43TA and 400? using probes 405 421 427 and 402 Yellow Springs Ohio USA) Recordings were made with a Watanabe recording system (Watanabe Instruments Corp Tokyo Japan)

MEASUREMENT PROCEDURE

All measurements were made with the naked infant placed in an incubator (AGA MK41 or MK241 AGA Medical Lidingo Sweden) The inflow of air to the incubator was kept at a constant level during the measurements in each infant and ranged between 8–15 l/min (cf 5) The ambient humidity was regulated as described previously (5) T_{skin} was registered from the skin areas where ER measurements were made T_{body} was recorded as deep rectal temperature and T_{amb} was measured in the central part of the incubator By regulating T_{amb}

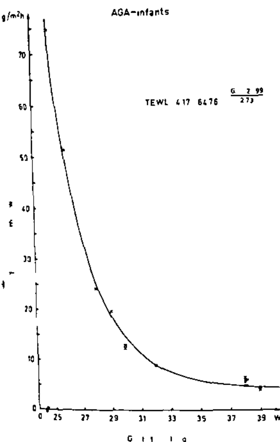


Fig 3 Transepidermal water loss (TEWL) in relation to gestational age (G A) AGA=appropriate for gestational age w=completed weeks of gestation

of life in newborn infants of different gestational ages There are large dissimilarities in the state of hydration (4) in the control of body water (11) and in the ability to compensate for disturbances in water balance (for review see 8) Further little is known about the magnitudes of the water losses to the environment and the factors that influence them This has resulted in wide divergences of opinion as to the amount of fluid to be supplied

The investigation of insensible water loss (IL) by Fanaroff et al (2) showed that this was very high in infants with a birth weight less than 1250 g No report appears to have been published concerning the relation between water losses and gestational age In order to separate the effects of intrauterine nutrition and degree of maturity only the latter has been dealt with in this study Thus only preterm infants appropriate for gestational age (AGA) were included

The present results elucidate the great differences in transepidermal water loss between preterm and full term infants and demonstrate an exponential relationship between gestational age and TEWL This may explain the clinical observation that infants with very low birth weights without noticeable extreme water losses sometimes succumb to hypertonic dehydration (2) although the ordinary amount of fluid per surface area is supplied Further the high transepidermal water loss may be one of the reasons for the more frequent occurrence of intracranial haemorrhage in preterm infants as this seems to be related to hyperosmolarity (18)

In order to avoid hyperosmolarity the use of larger amounts of fluid has been proposed but problems of overhydration are then frequent and patent ductus arteriosus may occur during the first week of life in the smallest infants (17)

It is clear from the exponential shape of TEWL gestational age curve (Fig 3) that differences in gestational age of 2–3 weeks might cause great changes in the transepidermal water loss which renders proper adjustments of the fluid supply to the infant difficult

From Tables 1 and 2 it is clear that a much higher ambient air temperature was necessary to keep the body temperature between 36 0 and 37 0°C in the infants born after a short gestational period As the ambient relative humidity was kept constant at 50% the vapour pressure in the ambient air ($P_{H_2O\ mb}$) was higher in these measurements The exponential curve would thus have been even steeper if the vapour pressure had been kept constant instead

The results of this study also explain the tendency for infants of very low gestational age to lose body heat despite a high ambient temperature The heat losses through eva

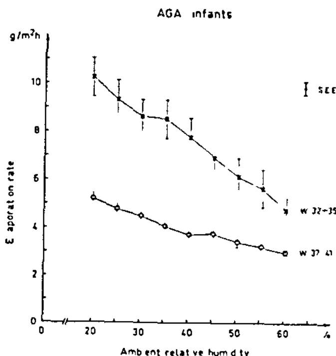


Fig. 1. The relation between evaporation rate (ER) and ambient relative humidity (RH_{amb}) in preterm and full term infants. S.E.E. = standard error of the estimate. AGA = appropriate for gestational age. *w = completed weeks of gestation.*

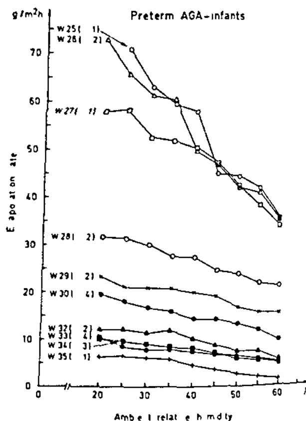


Fig. 2. The relation between evaporation rate (ER) and ambient relative humidity (RH_{amb}) in preterm newborn infants in different gestational age groups. AGA = appro*priate for gestational age. w = completed weeks of gesta*tion.

1b. *Evaporation rate at different ambient humidities in relation to gestational age*

In Fig. 2 the relation between ER at different RH_{amb} and gestational age is shown. The figure includes data from 12 infants born after 25 to 30 weeks of gestation and 10 infants born after 32 to 35 weeks. The ER at an RH_{amb} of 50% was 44 g/m²h for the infant born after 25 weeks, 13.2 g/m²h for the infants born after 30 weeks and 6.0 g/m²h for the infants born after 34 weeks. The susceptibility to changes in ambient humidity, as represented by the slope of each curve, was greater at lower gestational ages.

2. *Transepidermal water loss in relation to gestational age*

In the 32 infants of gestational ages of 25 to 39 weeks in whom estimations of TEWL (g/m²h) were made, this loss showed an inverse relationship to gestational age (Fig. 3). The relationship can be described by the following equation obtained by regression analysis:

$$TEWL = 4.17 + 64.76\ e^{-\frac{(G.A. - 4.99)}{73}} \qquad (2)$$

where G.A. is gestational age (weeks). The *residual standard deviation of the equation is* 2.72 ($n=32$, 28 degrees of freedom).

In infants with a gestational age of less *than 30 completed weeks the TEWL values* were considerably higher than in infants near or at term. Thus during the first day of life TEWL was 15 times higher in the infants born after 25 weeks of gestation than in the full term infants.

DISCUSSION

It is difficult to define what is an acceptable loss of weight and water during the first days

Proc Internat Conferen e on Biomedical transducers Paris 3–7/11 1975 part II p 71
15 Sedin G Hammarlund K Nilsson G E & Oberg P Å Evaporative water loss in full term and premature newborn infants *Abstract no 47 Sixth Nordic Congress of Perinatal Medicine* Oulu 15–17/9 1977
16 Sedin G Hammarlund K Nilsson G E & Oberg P Å The influence of environment activity and gestational age on evaporative water loss *Proc Annual Meeting of European Society for Pediatric Research* no 51 Turku 25–29/6 1978
17 Stevenson J G Fluid administration in the association of patent ductus complicating respiratory distress syndrom *J Pediatr* 90(?) ?57 1977
18 Thomas D B Hyperosmolality and intraventricular haemorrhage in premature babies *Acta Paediatr Scand* 65 4 9 1976

Submitted Feb ?8 1979
Accepted June 7 1979

(G S) Department of Paediatrics
University Hospital
S 750 14 Uppsala
Sweden

poration are very high especially at low ambient relative humidities (cf Fig 2) The fact that the water loss by evaporation from the skin was increased by 100% in infants born after a gestational period of 26 completed weeks when the ambient relative humidity was reduced from 60 to 20% emphasizes the enormous importance of keeping the air in the incubator well humidified

The high transepidermal water loss in preterm infants may be at least partly explained by their thin epidermal layer and their numerous superficial skin vessels Very little is known about other factors that may be involved in the passage of water through the skin of newborn preterm infants

CONCLUSIONS

This study shows that during the first day of life

1 There is a linear relation between the ambient humidity and the evaporation rate from the skin surface in preterm and fullterm infants

2 The evaporation rate from the skin is higher in preterm infants than in term infants

3 Susceptiblity to changes in ambient humidity increases with decreasing gestational age

4 The water loss by evaporation from the skin is increased by 100% in infants born after very short gestational periods when the ambient relative humidity is changed from 60 to 20%

5 There is an exponential relationship between transepidermal water loss and gestational age

ACKNOWLEDGEMENTS

This study was supported by grants from the Swedish Medical Research Council (19 X 04998) the Samaritan Foundation Stockholm the Forenade Liv Mutual Group Life Insurance Company Stockholm and the Medical Faculty of the University of Uppsala The authors wish to thank B Östmark for skilful laboratory and technical assistance S G Nilsson S G Norberg H Petersson and O Wistrand for expert technica assistance and L Grandin and C Odenjo for typin the manuscript The valuable help of B Bergman an her collaborators at the Neonatal Unit of the Universit Hospital Uppsala is also gratefully acknowledged

REFERENCES

1 Dubowitz L M S Dubowitz V & Goldberg C Clinical assessment of gestational age in the newbor infant *J Pediatr 77* 1 1970
2 Fanaroff A A Wald M Gruber H S & Klaus M H Insensible water loss in low birth weigh infants *Pediatrics 50* 236 1972
3 Finnstrom O Studies on maturity in newbor infants IX Further observations on the use o external characteristics in estimating gestational age *Acta Paediatr Scand 66* 601 1977
4 Friis Hansen B Changes in body water compart ments during growth *Acta Paediatr Scand* Suppl 110 1956
5 Hammarlund K Nilsson G E Öberg P Å & Sedin G Transepidermal water loss in newbor infants I Relation to ambient humidity and site o measurement and estimation of total transepiderma water loss *Acta Paediatr Scand 66* 553 1977
6 Hammarlund K Nilsson G E Öberg P Å & Sedin G Transepidermal water loss in newbor infants II Relation to activity and body tempera ture *Acta Paediatr Scand 68* 371 1979
7 Hammarlund K & Sedin G Evaporative wate loss in preterm newborn infants *Abstract no 19 Sixth European Congress of Perinatal Medicine* Vienna 29/8 – 1/9 1978
8 Heird W C Grebin B & Winters R W Th stabilization of disorders of water electrolyte an acid base metabolism in newborn infants under in tensive care *In* H Abramson (ed) *Resuscitatio of the newborn infant* The Mosby Company St Louis 1973 p 240
9 Hey E N & Katz G Evaporative water los in the newborn baby *J Physiol 200* 605 1969
10 Karlberg P & Priolisi A Clinical evaluation o similarities and dissimilarities between the two city surveys *In* F Falkner (ed) *Fundamentals o mortality risks during the perinatal period and in fancy* Monogr Paediatr 9 165 Karger Basel 1977
11 MacLaurin J C Changes in body water distribu tion during the first two weeks of life *Arch Di Child 41* 286 1966
12 Nilsson G E Measurement of water exchange through skin *Med Biol Eng Comput 15* 209 1977
13 Nilsson G E On the measurement of evaporative water loss Methods and clinical applications *Lin koping Studies in Science and Technology Disserta tions no 11* and *Linkoping University Medical Dis sertations no 48* Linkoping 1977
14 Nilsson G Sedin G & Öberg A A transducer for measurement of evaporation from the skin

Acta Pædiatr Scand 68 803–806 1979

ARTERIAL BLOOD PRESSURE ELEVATIONS DURING MOTOR ACTIVITY AND EPILEPTIC SEIZURES IN THE NEWBORN

H C LOU and B FRIIS HANSEN

From the Department of Neurology Roskilde Hospital and the Department of Neonatology Rigshospitalet Copenhagen Denmark

ABSTRACT Lou H C and Friis-Hansen B (Department of Neurology Roskilde Hospital and the Department of Neonatology Rigshospitalet Copenhagen Denmark) Arterial blood pressure elevations during motor activity and epileptic seizures in the newborn Acta Paediatr Scand 68 803 1979.—In nine distressed newborn infants mean aortic blood pressure and motor activity were recorded continuously during the first two or three days of life Six of the infants had been asphyxiated at birth the remainder having idiopathic respiratory distress only The results showed that mean arterial blood pressure varies synchronously with motor activity reaching maximum values much higher than previously suspected about 90 or 100 mmHg were recorded in several infants In three cases focal and/or generalized epileptic seizures occurred during the recording It was found that in these circumstances too blood pressure increases dramatically even if the motor component of the seizure is insignificant

KEY WORDS Hypertension motor activity epileptic seizures neonates intracranial bleedings

Recent evidence suggests that the arterial blood pressure may play a decisive role in the development of intraventricular bleeding in the newborn (1 2) Most reports on the arterial blood pressure in newborn have focused on the resting blood pressure However Moss et al (3) and Gupta et al (4) have found that feeding and crying is associated with blood pressure increases of up to 25 mmHg The effects of general motor activity as well as handling and caring procedures are not well documented and blood pressure changes during epileptic seizures have not been studied at all in the newborn The present study was designed to obtain such information

PATIENT GROUP

The patient group consisted of 9 consecutive infants with varying degrees of distress requiring monitoring with an intra arterial catheter 6 had been asphyxiated at birth

Table 1 *Patient group*

Patient no	Sex	Birth weight (g)	Gest age (weeks)	Apgar score		IRDS	MABP at rest (awake) mmHg			Maximum MABP mmHg		
				1 min	5 min		Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
133	M	880	8	5	6	−	45	50	60	75	90	95
138	M	1 700	39	9	10	+	35	35		55	55	
31	F	070	33	1		−	30	40		60	80	
58	M	040	3		9	+	45	55		55	85	
38	M	50	35	1	8	−	40	45	45	60	90	90
350	F	400	38	7	10	−	40	40		70	80	
	F	900	36	10	10	+	60	65	65	100	100	110
70	M	900	40	3	4	−	50	50		70	80	
53	F	3 000	39	4	6	−	50	50	40	100	75	80

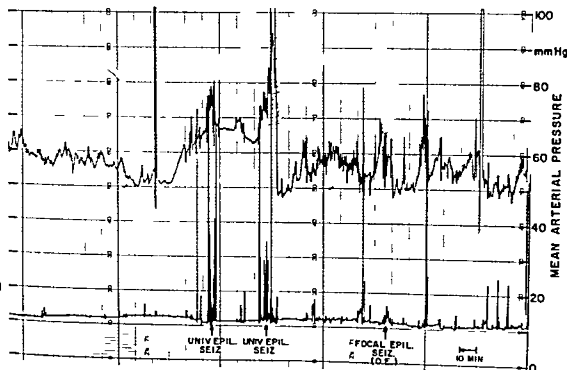


Fig 3 MABP increases from 50 to 95 mmHg are seen during generalized epileptic seizures (Patient 253)

were considerably higher than previously realized 55–110 mmHg (mean 86) All patients had marked increases synchronously with increases in motor activity (Fig 1) whether spontaneous (spont M A) or induced by the nursing procedure (suction (suct)) feeding handling observation procedure (obs proc) physiotherapy (phys) etc

In 3 of the patients (253 270 and 350) seizure activity was present during the recording Patient 350 had brief generalized tonic–clonic convulsions as well as focal myoclonic seizures in the right leg The EEG was characterized by sharp waves and spikes bitemporally and a background activity of 5–6 Hz with admixture of 9–10 Hz as well as 1½–3 Hz activity The siezures were associated with an increase in MABP from about 40 to 80 mmHg Patient 270 had periodic paroxysms of up to 6 sec duration with 4–5 Hz activity and spike potentials in the EEG These episodes were associated with apnoea and in some instances slight tonic convulsions of upper extremities as well as sharp increases in MABP (Fig 2)

Patient 253 had focal myoclonic jerks of the upper extremities (focal epil seiz) as well as a few generalized tonic–clonic convulsions (univ epil seiz) of short duration during the first day of life The convulsions ceased with phenobarbitone treatment Repeated EEGs were normal Fig 3 shows the very dramatic increase in MABP during seizures especially during one of the generalized seizures with MABP reaching values of about 95 mmHg

DISCUSSION

The principal finding of the present study has been the demonstration of the instability of MABP High values are reached during spontaneous motor activity manipulation of the infant and during seizures whether or not associated with motor phenomena The maximum MABP was in most cases about 100%

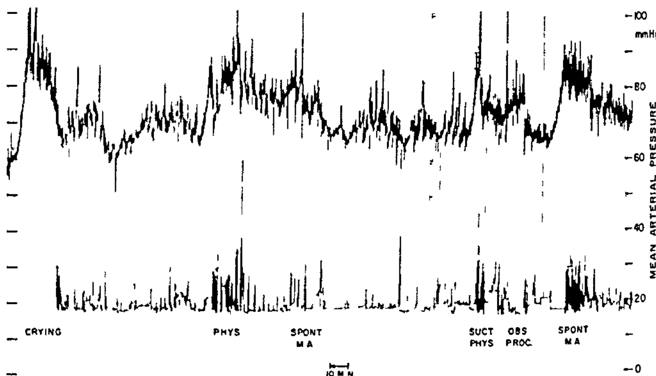


Fig 1 Increases of MABP from 60 or 65 mmHg to 100 mmHg are seen to occur synchronously (*upper curve*) with registrated motor activity (*lower curve*) (During the vigorous movements of crying the strain gauge is over stretched) (Patient 222)

(Apgar score below 7/1 min) and 3 had idiopathic respiratory distress syndrome 6 had a birth weight below 2 500 g (Table I)

METHODS

Intra aortic mean pressure was recorded continuously by means of an end hole umbilical artery catheter with the tip in the thoracic aorta The catheter was connected through a rigid polyethylene tube and a flushing valve to a transducer (Statham[8]) placed on the same level The arterial blood pressure was electronically integrated with a time constant of 10 sec and the mean arterial blood pressure (MABP) continuously recorded with a paper speed of 1 mm/min In this way artifacts were minimized The recording started 2–3 hours after birth and was continued throughout the first 2 or 3 days of life The motor activity of the neonate was recorded synchronously by means of a strain gauge tied around the left leg (Medimatic[8])

RESULTS

The principal findings are shown in the table The MABP at rest was in accordance with other studies found to be in the range of 30–60 mmHg (mean 45) during the first days of life However the maximum MABPs recorded

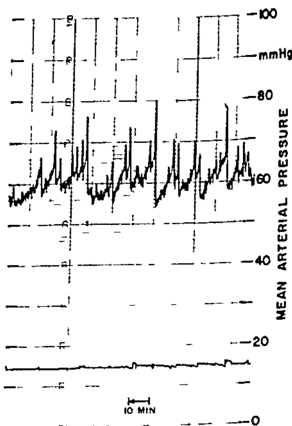


Fig 2 Sharp increases of MABP from 55 to 80 mmHg are observed during subtle seizures consisting of apnoea with or without accompanying myoclonic jerks of the upper extremities No motor activity is registered in the lower extremities (Patient 270)

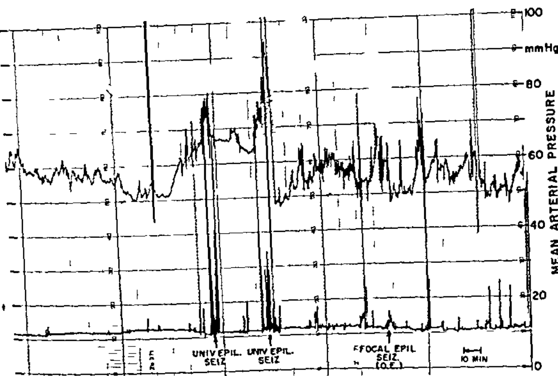


Fig 3 MABP increases from 50 to 95 mmHg are seen during generalized epileptic seizures (Patient 253)

ere considerably higher than previously alized 55–110 mmHg (mean 86) All pa ents had marked increases synchronously ith increases in motor activity (Fig 1) hether spontaneous (spont M A) or in uced by the nursing procedure (suction suct)) feeding handling observation proce ure (obs proc) physiotherapy (phys) etc

In 3 of the patients (253 270 and 350) seizure ictivity was present during the recording Patient 350 had brief generalized tonic–clonic convulsions as well as focal myoclonic sei zures in the right leg The EEG was char acterized by sharp waves and spikes bitempo-rally and a background activity of 5–6 Hz with admixture of 9–10 Hz as well as 1½–3 Hz ac tivity The siezures were associated with an increase in MABP from about 40 to 80 mmHg Patient 270 had periodic paroxysms of up to 6 sec duration with 4–5 Hz activity and spike potentials in the EEG These episodes were associated with apnoea and in some instances slight tonic convulsions of upper extremities as well as sharp increases in MABP (Fig 2)

Patient 253 had focal myoclonic jerks of the upper extremities (focal epil seiz) as well as a few generalized tonic–clonic convulsions (univ epil seiz) of short duration during the first day of life The convulsions ceased with phenobarbitone treatment Repeated EEGs were normal Fig 3 shows the very dramatic increase in MABP during seizures especially during one of the generalized seizures with MABP reaching values of about 95 mmHg

DISCUSSION

The principal finding of the present study has been the demonstration of the instability of MABP High values are reached during spon taneous motor activity manipulation of the infant and during seizures whether or not associated with motor phenomena The maxi mum MABP was in most cases about 100%

higher than MABP at rest and increases of 50% or more were recorded many times during the day in all patients. This effect is probably due to increased sympathetic tone.

The variability of MABP in the newborn is greater than previously suspected. The dramatic increases even in small prematures may be responsible for the often fatal intracranial bleeding.

It has been shown recently that such bleeding originates in the capillaries of the germinal matrix (1) and that the capillaries are unprotected by normal autoregulation in the distressed newborn (2). In addition the capillaries are not supported by the firm glial membranes of the more mature brain (5). Thus the delicate capillary wall of the distressed premature infants is completely exposed to the large increases in MABP seen even in the first days of life.

REFERENCES

1. Hambleton, G. & Wigglesworth, J. S.: Origin of intraventricular haemorrhage in the preterm infant. *Arch Dis Child 51*: 651, 1976.
2. Lou, H. C., Lassen, N. A. & Friis-Hansen, B.: Impaired autoregulation of cerebral blood flow in the distressed newborn. *J Pediatr 94*: 118, 1979.
3. Moss, A. J., Duffie, E. R. & Emmanouilides, G.: Blood pressure and vasomotor reflexes in the newborn infants. *Pediatrics 32*: 175, 1963.
4. Gupta, J. M. & Scopes, J. W.: Observations on blood pressure in newborn infants. *Arch Dis Child 40*: 637, 1965.
5. Prestige, M. C.: Axon and cell numbers in the developing nervous sytem. *Br Med Bull 30*: 107, 1974.

Submitted Feb. 23, 1979
Accepted June 8, 1979

(H. C. L.) Department of Neurology
Roskilde Hospital
DK-4000 Roskilde
Denmark

Acta Pædiatr Scand 68 807–811 1979

NEONATAL CONVULSIONS

Incidence and Causes in the Stockholm Area

MARGARETA ERIKSSON and ROLF ZETTERSTRÖM

From the Department of Paediatrics Karolinska Institute St Goran s Children s Hospital Stockholm Sweden

ABSTRACT Eriksson M and Zetterstrom R (Department of Paediatrics Karolinska Institute St Goran s Children s Hospital Stockholm Sweden) Neonatal Convulsions Incidence and Causes in the Stockholm Area Acta Paediatr Scand 68 807 1979 —The causes and short term prognoses of neonatal convulsions in infants less than four weeks of age were studied in 77 full term infants born in Stockholm in 1970–1976 In half of the infants (48%) hypoxia was considered to be the probable main etiology while infection and metabolic disease including hypoglycemia and hypocalcemia were the next commonest cause 12% for each condition The etiology was unknown in 29% of the infants although 15 of those 22 included in this group had other additional diagnoses The total mortality was 13% At one year of age 19 of the surviving 64 infants (30%) had severe psychomotor retardation Of 11 infants with normal mental development at 12 months of age 6 had cerebral palsy and 5 epileptic seizures Thirty four (53%) of the infants still had no signs of sequelae The poorest prognosis was found in the group with hypoxia as the main probable etiology The incidence of neonatal convulsions was 1 5 per 1 000 full term deliveries In a similar study from Gothenburg which was performed 10 years earlier the incidence was 3 7 per 1 000 Corresponding figures for perinatal mortality rate were 13 5 and 23 8

KEY WORDS Newborn convulsions etiology prognosis incidence

There is evidence that an increased concern for maternity and infant care has led to a reduced neonatal mortality and morbidity (8 22) In Sweden this has resulted not only in a decline in perinatal mortality but it has also been accompanied by a significant decrease in the incidence of cerebral palsy during the last decades (12) The decrease in cerebral palsy mainly relates to perinatally and neonatally acquired forms and has been attributed to better obstetric and neonatal care Against this background we decided to study another symptom which is frequently related to the quality of prenatal and neonatal and obstetric care—i e neonatal convulsions (7)

The reported incidence of neonatal convulsions varies considerably in different parts of the world Several prospective studies and review articles give figures ranging from 3 8 to 14 0 per 1000 deliveries (4 7 14 19 20) In Sweden an incidence of 3 7/1 000 term infants was reported from Gothenburg for 1960–1962 (23) The possible etiologies for convulsions and their relative frequencies also vary

In several recent studies the mortality rate and the incidence of sequelae in newborns with convulsions appear to have changed (15 18 20) Until 1970 the reported mortality was about 40% and one third of the surviving infants showed sequelae A lower mortality rate has been reported in later reviews (15–20%) on the other hand a large number of the surviving infants are handicapped (40%) The presence of sequelae correlates with the possible etiology of the seizures as well as with its severity and the age of the infant when convulsions develop The poorest prognosis has been found in infants with perinatal asphyxia and early symptoms (13 21)

MATERIAL AND METHODS

Information has been collected concerning 77 consecutive infants with neonatal convulsions who were seen at St Goran s Children s Hospital during a seven year period

higher than MABP at rest and increases of 50% or more were recorded many times during the day in all patients This effect is probably due to increased sympathetic tone

The variability of MABP in the newborn is greater than previously suspected The dramatic increases even in small prematures may be responsible for the often fatal intracranial bleeding

It has been shown recently that such bleeding originates in the capillaries of the germinal matrix (1) and that the capillaries are unprotected by normal autoregulation in the distressed newborn (2) In addition the capillaries are not supported by the firm glial membranes of the more mature brain (5) Thus the delicate capillary wall of the distressed premature infants is completely exposed to the large increases in MABP seen even in the first days of life

REFERENCES

1 Hambleton G & Wigglesworth J S Origin of intraventricular haemorrhage in the preterm infant *Arch Dis Child 51* 651 1976
2 Lou H C Lassen N A & Friis Hansen B Impaired autoregulation of cerebral blood flow in the distressed newborn *J Pediatr 94* 118 1979
3 Moss A J Duffie E R & Emmanouilides G Blood pressure and vasomotor reflexes in the newborn infants *Pediatrics 32* 175 1963
4 Gupta J M & Scopes J W Observations on blood pressure in newborn infants *Arch Dis Child 40* 637 1965
5 Prestige M C Axon and cell numbers in the developing nervous sytem *Br Med Bull 30* 107 1974

Submitted Feb 23 1979
Accepted June 8 1979

(H C L) Department of Neurology
Roskilde Hospital
DK 4000 Roskilde
Denmark

Acta Pædiatr Scand 68: 807–811, 1979

NEONATAL CONVULSIONS

Incidence and Causes in the Stockholm Area

MARGARETA ERIKSSON and ROLF ZETTERSTRÖM

From the Department of Paediatrics, Karolinska Institute, St Göran's Children's Hospital, Stockholm, Sweden

ABSTRACT. Eriksson, M. and Zetterström, R. (Department of Paediatrics, Karolinska Institute, St Göran's Children's Hospital, Stockholm, Sweden). Neonatal Convulsions. Incidence and Causes in the Stockholm Area. Acta Paediatr Scand, 68: 807, 1979.—The causes and short term prognoses of neonatal convulsions in infants less than four weeks of age were studied in 77 full term infants born in Stockholm in 1970–1976. In half of the infants (48%) hypoxia was considered to be the probable main etiology, while infection and metabolic disease including hypoglycemia and hypocalcemia were the next commonest cause, 12% for each condition. The etiology was unknown in 29% of the infants although 15 of those 22 included in this group had other additional diagnoses. The total mortality was 13%. At one year of age 19 of the surviving 64 infants (30%) had severe psychomotor retardation. Of 11 infants with normal mental development at 12 months of age 6 had cerebral palsy and 5 epileptic seizures. Thirty four (53%) of the infants still had no signs of sequelae. The poorest prognosis was found in the group with hypoxia as the main probable etiology. The incidence of neonatal convulsions was 1.5 per 1 000 full term deliveries. In a similar study from Gothenburg which was performed 10 years earlier the incidence was 3.7 per 1 000. Corresponding figures for perinatal mortality rate were 13.5 and 23.8.

KEY WORDS: Newborn, convulsions, etiology, prognosis, incidence

There is evidence that an increased concern for maternity and infant care has led to a reduced neonatal mortality and morbidity (8, 22). In Sweden, this has resulted not only in a decline in perinatal mortality, but it has also been accompanied by a significant decrease in the incidence of cerebral palsy during the last decades (12). The decrease in cerebral palsy mainly relates to perinatally and neonatally acquired forms and has been attributed to better obstetric and neonatal care. Against this background, we decided to study another symptom which is frequently related to the quality of prenatal and neonatal and obstetric care—i.e. neonatal convulsions (7).

The reported incidence of neonatal convulsions varies considerably in different parts of the world. Several prospective studies and review articles give figures ranging from 3.8 to 14.0 per 1000 deliveries (4, 7, 14, 19, 20). In Sweden, an incidence of 3.7/1 000 term infants was reported from Gothenburg for 1960–1962 (23). The possible etiologies for convulsions and their relative frequencies also vary.

In several recent studies the mortality rate and the incidence of sequelae in newborns with convulsions appear to have changed (15, 18, 20). Until 1970 the reported mortality was about 40% and one third of the surviving infants showed sequelae. A lower mortality rate has been reported in later reviews (15–20%); on the other hand, a large number of the surviving infants are handicapped (40%). The presence of sequelae correlates with the possible etiology of the seizures as well as with its severity and the age of the infant when convulsions develop. The poorest prognosis has been found in infants with perinatal asphyxia and early symptoms (13, 21).

MATERIAL AND METHODS

Information has been collected concerning 77 consecutive infants with neonatal convulsions who were seen at St Göran's Children's Hospital during a seven year period

Table 1 *Probable etiology of neonatal convulsions in 77 infants*

Probable main etiology	Infants No	%
Hypoxia	37	48
Infection	9	12
Metabolic hypoglycemia	5	6
Metabolic hypocalcemia	2	3
Other defined metabolic causes	2	3
Unknown	22	29
No additional diagnosis	7	
Unknown other disease	15	

(1970–1976) The infants came both from the referral area of its three maternity hospitals (33 976 deliveries) and from other pediatric hospitals for intensive care Full term infants (≥37 gestational weeks) with clonic or tonic seizures less than four weeks of age were included in the study Ten pre term infants presenting during the same period were not included because in some cases it was difficult to diagnose seizures in these infants Gestational age was calculated on the basis of external and neurological maturity characteristics

The following data were obtained from case records information on pregnancy delivery (including signs of fetal distress) Apgar score at 1 and 5 min blood glucose serum calcium and magnesium as well as other diagnoses and investigations relevant to the seizure disorder The infants were classified into different groups with regard to the most probable cause of the seizure The diagnosis of hypoglycemic and hypocalcemic convulsions was made when the criteria of a low blood glucose (1 7 mmol/l) serum calcium (1 9 mmol/l) and cessation of seizures upon administration of glucose or calcium were fulfilled (2 6)

Most infants were followed in a special high risk clinic up to the age of one year In a few cases information was obtained by interview and examination of records at the child health centre

RESULTS

The incidence of neonatal convulsions in our referral area (i e 49 of the 77 infants) was estimated as 1 5/1 000 full term deliveries Of the 77 full term infants 17 (22%) had a birth weight two standard deviations below the mean The corresponding figure for Sweden in 1974 was 2 2% (according to the birth register at the National Board of Health and Welfare)

Table 1 shows the distribution of the infants according to the most probable cause The 15 infants with an additional diagnosis include 4 with congenital malformations 3 with hyperbilirubinemia (bilirubin 270–500 mmol/l) 3 small for gestational age babies one neonatal polycythemia (packed red cell volume 76%) one maternal diabetes one hemolytic disea from Rh immunization and one neonatal hep titis The total mortality was 10/77 (13%)

The majority of infants had their first si zure before three days of age An early ons was common in asphyxia and a late onset w common in septicemia One third of the i fants had mild convulsions (i e less than fo convulsions each of less than 10 min dur tion) No difference in severity between tl groups was found except in otherwise norm infants with convulsions of unknown etiolog where multiple prolonged seizures were a ways noted

Table 2 shows the biochemical changes all infants grouped according to the most prol able etiology As can be seen low blood gl cose and serum calcium concentrations wel found in several infants with asphyxia A phyxia was also occasionally present in grou with other etiologies

The prognosis at one year of age which shown in Table 3 was found to be related t

Table 2 *Hypoglycemia and/or hypocalcemia in 77 infants with neonatal convulsions*

Probable main etiology	No	Blood glucose <1 7 mmol/l	Serum calcium <1 9 mmol/l
Hypoxia	37	12	3
Infection	9	1	1
Metabolic hypoglycemia	5	5	
Metabolic hypocalcemia	2		2
Other defined metabolic causes	2		1
Unknown no additional diagnosis	7	0	0
Unknown other disease	15	4	2

Table 3 *Follow up at one year of age of infants with neonatal convulsions*

Probable main etiology	No of infants	Mentally normal		No apparent sequelae	Obvious psycho-motor retardation	Died
		CP	Seizures			
Hypoxia	37	6	3*	16	6	6
Infection	9			3	4	2
Metabolic						
Hypoglycemia	5			2	3	
Hypocalcemia	[illegible]			2		2
Other	2					
Unknown						
No additional diagnosis	7		2	2	3	
Other disease	15			9	3	3

One infant in each group with severe retardation survived the neonatal period but died before one year of age
Only with high temperature

the severity of the convulsions In 11 children with a few short seizures presumably due to hypoxia no sequelae occurred Three how ever had febrile seizures starting at 6–9 months of age In other groups with mild con vulsions severe retardation was found in one child with meningitis and in one with symptomatic hypoglycemia Six children with cerebral palsy (varying severity of spastic diplegia) were considered to be mentally normal at one year of age

DISCUSSION

The incidence of neonatal convulsions was 1 5/1000 full term deliveries in our referral area for the years 1970–1976 This figure is considerably lower than the rates reported from other countries i e 3 7–14/1000 de liveries The incidence was found to be 3 7/1000 in Gothenburg which is Sweden s second largest city during the years 1960–1962 (23) One explanation for the difference between various studies may be related to the selection of patients especially with respect to the inclusion of pre term infants For ex ample Hopins (incidence 3 8 1000) included all infants with a birth weight above 1500 g while Rose & Lombreso studied those with a weight of 2500 g and more (14 19)

The reason for the low incidence of neonatal convulsions in our study and the declining in cidence in Sweden is probably multifactorial As mentioned in the introduction the low incidence in the Oxford area compared to Edinburgh and Manchester was tentatively attributed to better prenatal and obstetric area (7) In Sweden during the same period the de creases in perinatal mortality and in cerebral palsy a sequelae of perinatal complications have also been attributed to better perinatal care (9 12)

In this review the same grouping as in most other studies (i e according to the most probable etiology) has been used There is a demonstrable difference between these stud ies even if the grouping sometimes is difficult and may be open to discussion In the majority of the studies however perinatal hypoxia and birth trauma are the most common causes In our study we found that approximately 50% of the infants were subjected to perinatal hypoxia Lower relative incidences have been reported from England and the USA If how ever a comparison is made with respect to the total number of affected infants the rate of 13% due to asphyxia reported by Keen & Lee (15) from Manchester corresponds to 1 5/1000 deliveries which is still higher than the figure of 0 7/1000 in this Swedish review Dif ferences in the percentage due to metabolic disease have also been reported Symptomatic hypoglycemia however has been found in the same ralative incidence of 5–10% in most

Table 1 *Probable etiology of neonatal convulsions in 77 infants*

Probable main etiology	Infants No	%
Hypoxia	37	48
Infection	9	12
Metabolic hypoglycemia	5	6
Metabolic hypocalcemia	2	3
Other defined metabolic causes	2	3
Unknown	22	29
No additional diagnosis	7	
Unknown other disease	15	

(1970–1976) The infants came both from the referral area of its three maternity hospitals (33 926 deliveries) and from other pediatric hospitals for intensive care Full term infants (>37 gestational weeks) with clonic or tonic seizures less than four weeks of age were included in the study Ten pre term infants presenting during the same period were not included because in some cases it was difficult to diagnose seizures in these infants Gestational age was calculated on the basis of external and neurological maturity characteristics

The following data were obtained from case records information on pregnancy delivery (including signs of fetal distress) Apgar score at 1 and 5 min blood glucose serum calcium and magnesium as well as other diagnoses and investigations relevant to the seizure disorder The infants were classified into different groups with regard to the most probable cause of the seizure The diagnosis of hypoglycemic and hypocalcemic convulsions was made when the criteria of a low blood glucose (1 7 mmol/l) serum calcium (1 9 mmol/l) and cessation of seizures upon administration of glucose or calcium were fulfilled (2 6)

Most infants were followed in a special high risk clinic up to the age of one year In a few cases information was obtained by interview and examination of records at the child health centre

RESULTS

The incidence of neonatal convulsions in our referral area (i e 49 of the 77 infants) was estimated as 1 5/1 000 full term deliveries Of the 77 full term infants 17 (22%) had a birth weight two standard deviations below the mean The corresponding figure for Sweden in 1974 was 2 2% (according to the birth register at the National Board of Health and Welfare)

Table 1 shows the distribution of the infants according to the most probable cause The 15 infants with an additional diagnosis include 4 with congenital malformations 3 with hyperbilirubinemia (bilirubin 270–500 mmol/l) 3 small for gestational age babies one neonatal polycythemia (packed red cell volume 76%) one maternal diabetes one hemolytic disease from Rh immunization and one neonatal hepatitis The total mortality was 10/77 (13%)

The majority of infants had their first seizure before three days of age An early onset was common in asphyxia and a late onset was common in septicemia One third of the infants had mild convulsions (i e less than four convulsions each of less than 10 min duration) No difference in severity between the groups was found except in otherwise normal infants with convulsions of unknown etiology where multiple prolonged seizures were always noted

Table 2 shows the biochemical changes in all infants grouped according to the most probable etiology As can be seen low blood glucose and serum calcium concentrations were found in several infants with asphyxia Asphyxia was also occasionally present in groups with other etiologies

The prognosis at one year of age which is shown in Table 3 was found to be related to

Table 2 *Hypoglycemia and/or hypocalcemia in 77 infants with neonatal convulsions*

Probable main etiology	No	Blood glucose <1 7 mmol/l	Serum calcium <1 9 mmol/l
Hypoxia	37	12	3
Infection	9	1	1
Metabolic hypoglycemia	5	5	
Metabolic hypocalcemia	2		2
Other defined metabolic causes	2		1
Unknown no additional diagnosis	7	0	0
Unknown other disease	15	4	2

Table 3 *Follow up at one year of age of infants with neonatal convulsions*

Probable main etiology	No of infants	Mentally normal		No apparent sequelae	Obvious psycho-motor retardation	Died
		CP	Seizures			
Hypoxia	37	6	3*	16	6	6
Infection	9			3	4	2
Metabolic						
Hypoglycemia	5			2	3	
Hypocalcemia	–			[illegible]		[illegible]
Other	–					
Unknown						
No additional diagnosis	7		2	2	3	
Other disease	15			9	3	3

One infant in each group with severe retardation survived the neonatal period but died before one year of age
* Only with high temperature

the severity of the convulsions In 11 children with a few short seizures presumably due to hypoxia no sequelae occurred Three however had febrile seizures starting at 6–9 months of age In other groups with mild convulsions severe retardation was found in one child with meningitis and in one with symptomatic hypoglycemia Six children with cerebral palsy (varying severity of spastic diplegia) were considered to be mentally normal at one year of age

DISCUSSION

The incidence of neonatal convulsions was 1 5/1000 full term deliveries in our referral area for the years 1970–1976 This figure is considerably lower than the rates reported from other countries i e 3 7–14/1000 deliveries The incidence was found to be 3 7/1000 in Gothenburg which is Sweden s second largest city during the years 1960–1962 (23) One explanation for the difference between various studies may be related to the selection of patients especially with respect to the inclusion of pre term infants For example Hopins (incidence 3 8 1000) included all infants with a birth weight above 1500 g while Rose & Lombreso studied those with a weight of 2500 g and more (14 19)

The reason for the low incidence of neonatal convulsions in our study and the declining incidence in Sweden is probably multifactorial As mentioned in the introduction the low incidence in the Oxford area compared to Edinburgh and Manchester was tentatively attributed to better prenatal and obstetric area (7) In Sweden during the same period the decreases in perinatal mortality and in cerebral palsy a sequelae of perinatal complications have also been attributed to better perinatal care (9 12)

In this review the same grouping as in most other studies (i e according to the most probable etiology) has been used There is a demonstrable difference between these studies even if the grouping sometimes is difficult and may be open to discussion In the majority of the studies however perinatal hypoxia and birth trauma are the most common causes In our study we found that approximately 50% of the infants were subjected to perinatal hypoxia Lower relative incidences have been reported from England and the USA If however a comparison is made with respect to the total number of affected infants the rate of 13% due to asphyxia reported by Keen & Lee (15) from Manchester corresponds to 1 5/1000 deliveries which is still higher than the figure of 0 7/1000 in this Swedish review Differences in the percentage due to metabolic disease have also been reported Symptomatic hypoglycemia however has been found in the same ralative incidence of 5–10% in most

studies except in that of Hopkins (14) from Australia where it was demonstrated in one third of the cases However it remains unclear whether the criteria of symptomatic hypoglycemia which were used by Hopkins were the same as those used by other workers (2) Another explanation of the discrepancy might be a difference in the number of preterm infants and variations in feeding habits during the years concerned We found symptomatic hypoglycemia in association with both asphyxia and infection but in these instances the seizures did not respond to glucose administration If these infants are included hypoglycemia was found in almost one third of the coses i e in the same percentage as found by Hopkins (14) In studies from Scandinavia it has been found that asymptomatic hypoglycemia very often is associated with a number of pathological conditions in newborn infants and that only a minority of the infants with hypoglycemia develop convulsions (10 11 16)

A high incidence of hypocalcemia as a possible cause of neonatal convulsions has been reported from England and the USA (6 18 19) It is possible however that the requirement of cessation of convulsions upon administration of calcium was not mandatory in all cases In our study we did not find a low serum magnesium in a single case as a possible explanation of failure to respond to calcium administration However a study by Cockburn et al (6) showed that at the time of convulsions low calcium values could be found in the CSF moreover the establishment of equilibrium between the serum and the CSF could take some time after the administration of calcium consequently a prompt cessation of convulsions could not be expected Even if the few cases of a low serum calcium in the other groups are taken into consideration the absolute as well as the relative frequencies are low In the Edinburgh study (4) with an incidence of 14 0/1 000 live births disturbances in calcium or magnesium metabolism were thought to be the causative factor in 55% of the cases This in turn was suggested to be due to a higher phosphate content in cow s milk in comparison with breast milk since convulsions were less often seen in infants fed on a modified cow s milk formula with a low phosphate content (6)

Infections have been found to occur in about 10% of all studies of convulsions in which cases meningitis has usually been present The group with unknown etiology will naturally vary in different studies Some infants having a familial type of convulsion with a favourable prognosis have been included in this group (3 5)

The prognosis of neonatal convulsions has been investigated in a limited number of follow up studies (7 15 18 20) It will of course vary with the etiology Before 1970 a total early mortality of 40% sequelae in 19% and normal development in 41% of the infants were reported More recent studies have shown a lower mortality but also a higher percentage of survivors with sequelae while the percentage of infants with normal development has remained fairly constant (53%) We found an overall mortality of 13% and handicaps at the age of one year to be 53% Minor abnormalities may however be diagnosed later and long term follow up is important It is also important as mentioned earlier to analyse the figures in individual groups

A high mortality rate (43%) and sequelae in half of the surviving children has been reported in convulsions associated with asphyxia (15 21) We found a lower mortality rate (14%) but on the other hand more children with handicaps (41%) thus making the total morbidity about the same The unfavourable prognosis of convulsions in infants with asphyxia has been observed earlier in a Swedish study of children born 1943–1949 in which eight of nine children with convulsions had sequelae (13) The poorest prognosis is probably seen in this group with asphyxia and seizures (21)

In the two cases we found with hypocalcemia the prognosis was good as others have

also noted In hypoglycemia on the other hand the prognosis varies In a follow up of children with hypoglycemia the poorest prognosis was found in children with convulsions (10 11 16) Three of five children in our follow up were severely retarded We also found hypoglycemia as an additional symptom to be a bad prognostic sign in the other groups

One important finding is that convulsions per se in association with infections do not however lead to more sequelae than was reported in a follow up of children with neonatal septicemia (1) As others have also demonstrated the short term prognosis is favourable in infants with convulsions of unknown etiology except for a tendency to seizures later in life (3 5)

The incidence of neonatal convulsions in full term live births was much lower in this study of infants born 1970–1976 (1 5/1000) than in a similar study from Gothenburg from 1960–1962 with an incidence of 3 7/1 000 (23) The decrease may be attributed to improved obstetric and neonatal care The difference between the incidence of neonatal convulsions in the Gothenburg and Stockholm series is rather similar to corresponding figures for perinatal mortality rate which was 23 8 in Gothenburg 1960–1962 and 13 5 in Stockholm 1970–1976

Even if neonatal convulsions are still a sign which may indicate a serious condition with a high morbidity and risk for later sequelae the mortality rate is lower than in previous studies The children in this study however are still very young and it is important to have a long term follow up with respect to later and less serious sequelae

REFERENCES

1 Alfvén G Bergqvist G Bolme P & Eriksson M Longterm follow up of neonatal septicemia *Acta Paed at Scand* 67 769 1978

2 Beard A Cornblath M Gentz J Kellum M Persson B Zetterstrom R & Haworth J C Neonatal hypoglycemia A discussion *J Ped atr* 79 314 1971

3 Bjerre I & Corelius E Benign familial neonatal convulsions *Acta Paed atr Scand* 57 557 1968

4 Brown J K Convulsions in the newborn period *Dev Med Child Neurol* 15 823 1973

5 Carton D Benign familial neonatal convulsions *Neuropaediatrie* 9 167 1978

6 Cockburn F Brown J K Belton N R & Forfar J O Neonatal convulsions associated with primary disturbance of calcium phosphorus and magnesium metabolism *Arch Dis Child* 48 99 1973

7 Dennis J Neonatal convulsions aetiology late neonatal status and long term outcome *Dev Med Child Neurol* 20 143 1978

8 Elliot K & O Connor M *Major Mental Handicap and Costs of Prevention* Ciba Foundation Symposium 59 (New Series) Elsevier Excerpta Medica Amsterdam 1978

9 Ericson A Eriksson M & Zetterstrom R Analysis of perinatal mortality rate in the Stockholm area *Acta Paediatr Scand* In press

10 Fluge G Neurological findings at follow up in neonatal hypoglycaemia *Acta Paediatr Scand* 64 6 9 1975

11 Gentz J Persson B & Zetterstrom R On the diagnosis of symptomatic neonatal hypoglycemia *Acta Paediatr Scand* 58 449 1969

12 Hagberg B Hagberg G & Olow I The changing panorama of cerebral palsy in Sweden 1954–1970 *Acta Paediatr Scand* 64 187 1975

13 Hellstrom B & Jonsson B Late prognosis in asphyxia neonatorum *Acta Paediatr Scand* 4? 398 1953

14 Hopkins I Seizures in the first week of life A study of aetiological factors *Med J Aust* 2 647 197?

15 Keen J H & Lee D Sequelae of neonatal convulsions *Arch Dis Child* 48 54 1973

16 Koivisto M Blanco-Sequeiros M & Krause U Neonatal symptomatic and asymptomatic hypoglycaemia A follow up study of 151 children *Dev Med Child Neurol* 14 603 1972

17 McDonald R Purulent meningitis in newborn babies *Clin Pediatr (Phila)* 11 450 1972

18 McInerny T & Schubert W Prognosis of neonatal seizures *Am J Dis Child* 117 ?61 1969

19 Rose A & Lombroso C Neonatal seizure states A study of clinical pathological and electroencephalographic features in 137 full term babies with a long term follow up *Pediatrics* 45 404 1970

20 Volpe J Neonatal seizures *N Engl J Med* 289 413 1973

21 Volpe J Perinatal hypoxic ischemic brain injury *Pediatr Clin North Am* ?3 383 1976

22 Wallace H Status of infant and perinatal morbidity and mortality *Public Health Rep* 93 386 1978

23 Zetterstrom R Neonatal chemistry *In* H E Whipple & J A Sterling (eds) *Fetal and Infant Liver Function and Structure* Ann NY Acad Sci 111 New York 1963 p 537

Submitted March 15 1979
Accepted April 6 1979

(M E) Department of Paediatrics
St Goran s Children s Hospital
S 11 81 Stockholm Sweden

Acta Pædiatr Scand 68 813–817 1979

SODIUM EXCRETION IN RELATION TO SODIUM INTAKE AND ALDOSTERONE EXCRETION IN NEWBORN PRE TERM AND FULL TERM INFANTS

A APERIA O BROBERGER P HERIN and R ZETTERSTROM

From the Department of Paediatrics Karolinska Institutet
St Goran s Children s Hospital and Huddinge Hospital Stockholm Sweden

ABSTRACT Aperia A Broberger O Herin P and Zetterstrom R (Department of Paediatrics Karolinska Institutet S t Goran s Children s Hospital and Huddinge Hospital Stockholm Sweden) Sodium excretion in relation to sodium intake and aldosterone excretion in newborn pre term and full term infants Acta Paediatr Scand 68 813 1979 —The importance of aldosterone for the control of salt balance has been examined in pre term infants (gestational age 28–34 weeks) and in full term infants The post natal age has varied from 2–21 days Eight hour urinary specimens have been analysed with regard to sodium potassium and aldosterone The daily sodium intake has been recorded following determination of milk intake and analyses of sodium in breast milk Due to variations of sodium content of breast milk the daily sodium intake in pre term infants was lower than in full term infants during the first 10 days of life The sodium excretion was significantly higher in pre-term infants than in full term infants during the fist six days of life During the first week of life the sodium balance is negative in pre-term infants and positive in full term infants Aldosterone excretion is high during the first week of life and increases further from the ?nd to the 3rd week of life in both pre-term and full term infants The correlation between aldosterone excretion and urinary potassium/sodium quotient is 0 87 in full term infants 0 57 in pre-term infants aged 13– 0 days and does not exist in pre term infants aged 2–10 days It is suggested that the high sodium excretion in newborn pre term infants can in part be explained by an unresponsiveness to aldosterone at this developmental stage

KEY WORDS Neonatal Na balance neonatal aldosterone excretion renal maturation aldosterone responsi eness renal function in pre term infants renal function in full term infants

Newborn infants have a blunted natriuretic response to salt loading (3) which suggests a reduced tolerance to salt in the neonatal period It has therefore been recommended that the daily sodium intake administered orally or parenterally to newborn infants should be kept lower than the daily sodium intake to older children This recommendation has been supported by the assumption that breast milk during the entire period of lactation contains about 5 mmol/l of Na (18) There are however several observations mainly in pre term infants that are at variance with the recommendation that the daily sodium intake should not exceed the expected sodium intake when infants are fed breast milk containing 5 mmol/l of Na Newborn pre term infants receiving parenteral fluid with a sodium concentration of 10 and 20 mmol/l consistently develop a negative sodium balance (4) Newborn full term infants receiving parenteral fluids develop a negative sodium balance when the sodium concentration of the fluid is 10 mmol/l but generally not when it is 20 mmol/l Newborn pre term infants are reported to have a higher basal sodium excretion than newborn full term infants (5 6 13) and they frequently develop hyponatremia (7)

This study has been designed to describe the postnatal changes in sodium balance in pre term and full term infants with an uncomplicated postnatal course A record has been kept of the sodium intake and output In order

Dedicated to Andrea Prader a great scientist in the field of paediatrics on the occasion of his 60th anniversary

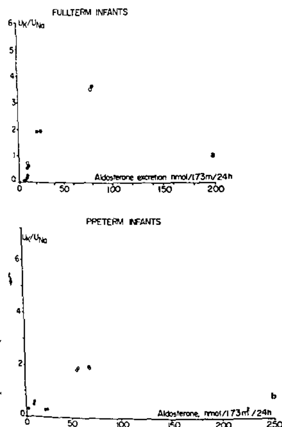
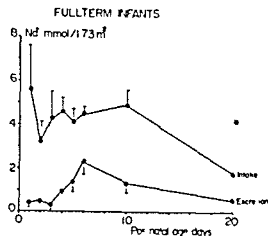


Fig 3a and b Relationship between aldosterone excretion and urinary K /Na ratio in full term infants (*a*) and pre term infants (*b*) I infants with gestational age 28–34 weeks and aged 1–16 days that are not reported in previous figures and tables are included in *b* Filled circles represent infants aged 1–10 days and unfilled circles infants aged 1 –.1 days

In newborn full term infants (Fig 1a) the urinary sodium excretion was relatively low during the second to fourth day of life increased slightly but insignificantly towards the end of the first week of life and then fell within the same range at three weeks of life as at two to four days of life The sodium concentration of breast milk was four times higher during the first 10 days after delivery than later during lactation (2) This resulted in a relatively high sodium intake during the first 10 days of life During the same period the intake of sodium in daytime well exceeded the excretion in the full term infants In pre term infants urinary sodium excretion was relatively high during the first days of life (Fig 1b) It gradually fell during the first week of life and reached the range observed in full term infants at the age of nine or ten days The differences between pre term and full term infants were significant during day 3–4 ($p<0.001$) and day 5–6 ($p<0.01$) All pre term infants included in this study received pooled breast milk during the first 10 days of life The sodium content of this milk averaged 5 mmol/l and the sodium intake was therefore considerably lower in pre term than full term infants In pre term infants the sodium excretion in daytime exceeded the sodium intake in daytime during the first five days of life At three weeks of age the sodium intake in daytime exceeded the sodium excretion in daytime to about the same extent as in full term infants

The postnatal changes in aldosterone excretion in pre term and full term infants are illustrated in Fig 2 During the first three weeks of age the average aldosterone excretion in pre term and full term infants ranged from 18 to 105 μmol/1 73 m B S A /day which is higher than in adults where the normal range in this laboratory is 5 5 to 33 μmol/1 73 m B S A /day The postnatal pattern for aldosterone excretion was similar in pre term and full term infants The excretion of aldosterone was generally higher in pre term than in full term infants but the difference was never of more than borderline significance $p>0.8$ during day 3–4 $0.1<p>0.05$ during day 5–12 Aldosterone excretion was highest at 3 weeks of age

It is well established that aldosterone affects tubular sodium and potassium transport in opposite directions An increase in the urinary potassium sodium quotient has therefore generally been interpreted as secondary to an effect of aldosterone on the tubular transport mechanism (10) In newborn full term infants there is a good correlation ($r=0.84$) between aldosterone excretion and urinary potassium sodium quotient (Fig 3a) The relationship is the same in full term infants aged 0–10 days as in full term infants aged 11–20 days In pre term infants aged 0–10 days there is no rela



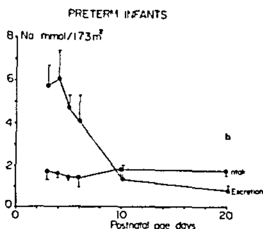


Fig 1 a and b Average hourly Na excretion and Na intake at the time of the study in full term infants aged 1–21 days (*a*) and in pre term infants aged 3–21 days (*b*) Each filled circle represents the average of 4–9 observations The bars represent S E M

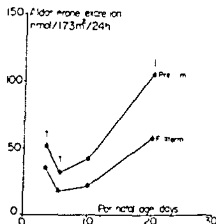


Fig 2 Postnatal changes in aldosterone excretion in pre term and full term infants Each filled circle represents the average of 4–10 observations The bars represent S E M

to elucidate some possible mechanisms responsible for the control of sodium balance in the neonatal period the aldosterone excretion was also determined in most of the infants studied

MATERIAL AND METHODS

35 pre term and 45 full term infants were studied on the Neonatal Ward of St Goran s Children s Hospital and in the Obstetric Ward of St Erik s Hospital

In the pre term infants the gestational age ranged from 28 to 34 weeks The postnatal course in these infants was regarded as uncomplicated since there was no need of assisted respiration antibiotic treatment for infection or exchange transfusion All pre term infants were kept in their incubators during the study They were fed via a gastric tube

In the full term infants the gestational age ranged from 39 to 41 weeks and the post natal course was uncomplicated The studies were carried out in the Obstetric Ward and the infants were nursed by their mothers

No mother was treated with diuretics during pregnancy

The gestational age of all infants was determined using the method of Dubowitz et al (9)

Urine was collected for eight to nine hours from each infant by spontaneous voiding into a plastic bag A tube was tightly fitted into the bag which allowed emptying of the bag without removing it Urine collection was started and completed immediately after a voiding In each case the urine collection was started between 7 30 and 9 30 a m During the study the infants were fed as usual either by a gastric tube (pre term infants) or by breast feeding (full term infants)

The infants were either fed breast milk from their mothers (full term infants) or pooled breast milk from mothers in a later stage of lactation (pre term infants) The milk intake from 8 a m to 4 p m (in general 3 meals) was recorded The sodium content of the milk was analysed in each individual case since it has been found to be higher during the first 2 weeks after delivery than later during lactation (2) The milk intake of the full term infants was assessed by weighing them immediately before and after each meal The average hourly sodium intake at the time of the study was calculated

Analyses of sodium and potassium in the urine were made with a flame photometer Analysis of aldosterone in the urine was made with a radioimmuno assay method (21)

Statistical analysis has been carried out with Student s *t* test

RESULTS

Fig 1 a and b illustrate the average Na^+ intake and output during the time of the study in infants aged three four five six eight to ten and 18–21 days

natal period (20) Studies in fetal guinea pigs have suggested that aldosterone is one factor of importance for the induction of this enzyme (11) It is of interest in this connection that the significant increase in aldosterone excretion which occurs from the first to the third week of life is not associated with a further fall in urinary sodium excretion and coincides with a period of rapid increase in the glomerular filtration rate (12) i e an increase of the work load to the renal tubules

REFERENCES

1 Aperia A & Broberger U β microglobulin an indicator of renal tubular maturation and dysfunction in the newborn *Acta Paediatr Scand* In press

2 Aperia A Broberger O Herin P & Zetterstrom R Salt content in human breast milk during the three first weeks after delivery *Acta Paediatr Scand* In press

3 Aperia A Broberger O Thodenius K & Zetterstrom R Renal response to an oral sodium load in newborn full term infants *Acta Paediatr Scand* 61 670 1972

4 Aperia A Broberger O Thodenius K & Zetterstrom R Renal control of sodium and fluid balance in newborn infants during intravenous maintenance therapy *Acta Paediatr Scand* 64 725 1975

5 Arant B S Developmental patterns of renal functional maturation compared in the human neonate *J Pediatr* 92 705 1978

6 Broberger U & Aperia A Renal function in idiopathic respiratory distress syndrome *Acta Paediatr Scand* 67 313 1978

7 Chance G W Radde I C Willis D M Roy R N Park E & Ackerman I Postnatal growth of infants of <1 3 kg birth weight Effects of metabolic acidosis of caloric intake and of calcium sodium and phosphate supplementation *J Pediatr* 91 787 1977

8 Dillon M J Gillin M E A Ryness J M & de Swiet M Plasma renin activity and aldosterone concentration in the human newborn *Arch Dis Child* 51 537 1976

9 Dubowitz L M S Dubowitz V & Goldberg C Clinical assessment of gestational age in the newborn infant *J Pediatr* 77 1 1970

10 Ganong W F & Mulrow P J Rate of change in sodium and potassium excretion after injection of aldosterone into the aorta and renal artery of the dog *Am J Physiol* 195 337 1958

11 Geloso J P Delaval E Geloso-Meyer A Lelievre M Enzyme development and functional differentiation of fetal rat kidney In *Ontogenesis of renal function 27th International Congress of Physiological Science* Paris 1977

12 Guignard J P Torrado A Dacunha O & Gauthier E Glomerular filtration rate in the first three weeks of life *J Pediatr* 87 268 1975

13 Honour J W Valman H B & Shackleton C G L Aldosterone and sodium homeostasis in full term infants *Acta Paediatr Scand* 66 103 1977

14 Jorgensen P L The role of aldosterone in the regulation of (Na + K)-ATP ase in rat kidney *J Steroid Biochem* 3 181 1972

15 Krans M Krecek J & Popp M The development of aldosterone production by the adrenal glands of normally and prematurely weaned rats *Biol Neonat* 11 338 1967

16 Kowarski A Katz H & Migeon C J Plasma aldosterone concentration in normal subjects from infancy to adulthood *J Clin Endocrinol Metab* 38 489 1974

17 Merlet Benichou C & de Rouffignac D Renal clearance studies in fetal and young guinea pigs effect of salt loading *Am J Physiol* 232 178 1977

18 Renner F *Milch und Milchprodukte in der Ernährung des Menschen* Volkswirtschaftliches Verlag GmbH Kempfen—Verlag Th Mann OHG Hildesheim 1974

19 Roy R N Chance G W Radde I C Hill D E Willis D M & Sheepers J Late hyponatremia in very low birth weight infants (<1 3 kg) *Pediat Res* 10 526 1976

20 Schmidt V & Horster M Na K activating ATP ase activity maturation in rabbit nephron segments dissected in vitro *Am J Physiol* 233 F55 1977

21 Sealey J E Buhler F R Laragh J H Manning E L & Brunner H R Aldosterone excretion *Circ Res* 31 367 1972

22 Siegel S R Fisher D A & Oh W Serum aldosterone concentrations related to sodium balance in the newborn infant *Pediatrics* 53 410

Submitted Jan 25 1979
Accepted May 4 1979

(A A) Department of Paediatrics
St Goran s Children s Hospital
S 11281 Stockholm
Sweden

tionship between the aldosterone excretion and the urinary potassium sodium quotient In pre term infants aged 10–20 days the urinary potassium sodium quotient is consistently higher than in younger pre term infants and there is a correlation between aldosterone excretion and the urinary potassium sodium quotient (r=0 57)

DISCUSSION

The findings of the present study explain the susceptibility of pre term infants to hyponatremia (19) During the first week of life pre term infants have both larger urinary sodium losses and a lower sodium intake than in full term infants The relatively low salt intake of pre term infants during the first week of life has previously been overlooked when the salt balance of newborn infants has been discussed Yet most pre term infants have been fed with pooled breast milk having a sodium concentration of 5 mmol/l or with a formula having a low sodium concentration It has however been reported that sodium supplementation to pre term infants on this diet results in a significant increase of body length (7) This finding together with the above mentioned observations of the frequently occuring negative salt balance in pre term infants (4 13 22) strongly suggests that pre term infants who for some reason cannot be wholly fed with breast milk from their mothers should at least during the first two weeks of life receive sodium supplements so that the daily sodium intake/kg b w corresponds to the sodium intake of breast fed term infants of similar postnatal age

High neonatal aldosterone production has previously been observed (8 13 16 22) in man as well as in other species (15) The neonatal aldosterone production does not primarily appear to be monitored by changes in salt balance During the first week of life there is a slight fall in aldosterone excretion which can be explained by a high sodium intake in the full term infants but this also occurred in pre term infants who had an inadequate sodium intake The aldosterone production appears to increase from the first to the third week of life not only in the newborn pre term infants who are in negative but also in the newborn full term infants who are in positive sodium balance Since the postnatal pattern for aldosterone excretion is almost identical in newborn pre term and full term infants birth appears to be an important regulator of aldosterone productionin early postnatal life

High sodium excretion in pre term infants has been reported in several previous studies (4 5 6 13 22) Since the glomerular filtration rate is lower in pre term than in full ter infants (5) a high sodium excretion in nev born pre term infants must be attributed to relatively low tubular reabsorption of sodiur The 34th to the 36th gestational week appea to be a period of rapid development for ren tubular transport mechanism The fraction reabsorption of β microglobulin (1) as well of glucose and amino acids (5) increase adult levels during this period Experiment studies in guinea pigs (17) have demonstrat that the fractional reabsorption of sodiu is low during fetal life and increases rapid at term It has been suggested that the chang in sodium excretion in guinea pigs are at lea partly monitored by aldosterone In the pr sent study it has been shown that aldosteror excretion is at least as high in newborn pr term infants with a high sodium excretion in newborn full term infants with a low sodiu excretion The lack of relationship betwe aldosterone excretion and the urinary pota sium sodium quotient in newborn pre term i fants does however strongly suggest that infants born before the 34th gestational weel the renal tubular sensitivity for aldosterone low This could be due either to a lack of r ceptors for aldosterone or to a deficiency the sodium transporting enzymes sodiu potassium activated ATP ase which ald sterone will affect (14) Experimental studie in rabbits have shown that the ATP ase act vity is low in all tubular segments in the nec

natal period (20) Studies in fetal guinea pigs have suggested that aldosterone is one factor of importance for the induction of this enzyme (11) It is of interest in this connection that the significant increase in aldosterone excretion which occurs from the first to the third week of life is not associated with a further fall in urinary sodium excretion and coincides with a period of rapid increase in the glomerular filtration rate (12) i e an increase of the work load to the renal tubules

REFERENCES

1 Aperia A & Broberger U β microglobulin an indicator of renal tubular maturation and dysfunction in the newborn *Acta Paediatr Scand* In press
2 Aperia A Broberger O Herin P & Zetterstrom R Salt content in human breast milk during the three first weeks after delivery *Acta Paediatr Scand* In press
3 Aperia A Broberger O Thodenius K & Zetterstrom R Renal response to an oral sodium load in newborn full term infants *Acta Paediatr Scand 61* 6 0 197?
4 Aperia A Broberger O Thodenius K & Zetterstrom R Renal control of sodium and fluid balance in newborn infants during intravenous maintenance therapy *Acta Paediatr Scand 64* 725 1975
5 Arant B S Developmental patterns of renal functional maturation compared in the human neonate *J Pediat* 9? 705 1978
6 Broberger U & Aperia A Renal function in idiopathic respiratory distress syndrome *Acta Paediatr Scand* 67 313 1978
7 Chance G W Raddle I C Willis D M Roy R N Park E & Ackerman I Postnatal growth of infants of <1 3 kg birth weight Effects of metabolic acidosis of caloric intake and of calcium sodium and phosphate supplementation *J Pediatr 91* 787 1977
8 Dillon M J Gillin M E A Ryness J M & de Swiet M Plasma renin activity and aldosterone concentration in the human newborn *Arch Dis Child 51* 537 1976
9 Dubowitz L M S Dubowitz V & Goldberg C Clinical assessment of gestational age in the newborn infant *J Pediatr 77* 1 1970
10 Ganong W F & Mulrow P J Rate of change in sodium and potassium excretion after injection of aldosterone into the aorta and renal artery of the dog *Am J Physiol 195* 337 1958
11 Geloso J P Delaval F Geloso-Meyer A Lelievre M Enzyme development and functional differentiation of fetal rat kidney In *Ontogenesis of renal function 27th International Congress of Physiological Science* Paris 1977
12 Guignard J P Torrado A Dacunha O & Gauthier E Glomerular filtration rate in the first three weeks of life *J Pediatr* 87 68 1975
13 Honour J W Valman H B & Shackleton C G L Aldosterone and sodium homeostasis in full term infants *Acta Paediatr Scand 66* 103 1977
14 Jorgensen P L The role of aldosterone in the regulation of (Na +K) ATP ase in rat kidney *J Steroid Biochem 3* 181 197?
15 Krans M Krecek J & Popp M The development of aldosterone production by the adrenal glands of normally and prematurely weaned rats *Biol Neonat 11* 338 1967
16 Kowarski A Katz H & Migeon C J Plasma aldosterone concentration in normal subjects from infancy to adulthood *J Clin Endocrinol Metab 38* 489 1974
17 Merlet Benichou C & de Rouffignac D Renal clearance studies in fetal and young guinea pigs effect of salt loading *Am J Physiol 232* 178 1977
18 Renner E *Milch und Milchprodukte in der Ernahrung des Menschen* Volkswirtschaftliches Verlag GmbH Kempfen—Verlag Th Mann OHG Hildesheim 1974
19 Roy R N Chance G W Radde I C Hill D E Willis D M & Sheepers J Late hyponatremia in very low birth weight infants (<1 3 kg) *Pediatr Res 10* 526 1976
20 Schmidt V & Horster M Na K activating ATP ase activity maturation in rabbit nephron segments dissected in vitro *Am J Physiol 233* F55 1977
21 Sealey J E Buhler F R Laragh J H Manning E L & Brunner H R Aldosterone excretion *Circ Res 31* 367 197
22 Siegel S R Fisher D A & Oh W Serum aldosterone concentrations related to sodium balance in the newborn infant *Pediatrics 53* 410

Submitted Jan 25 1979
Accepted May 4 1979

(A A) Department of Paediatrics
St Goran s Children s Hospital
S 11281 Stockholm
Sweden

Acta Pædiatr Scand 68 819–823 1979

GROUP B STREPTOCOCCAL COLONIZATION OF PREGNANT WOMEN AND THEIR NEONATES

Epidemiological Study and Controlled Trial of Prophylactic Treatment of the Newborn

P GERARD M VERGHOTE D HULST A BACHY and G DUHAUT

From the Department of Neonatal Medicine the Department of Obstetrics and the Laboratory of Microbiology Clinique Notre Dame Charleroi Belgium

ABSTRACT Gerard P Verghote-D Hulst M Bachy A and Duhaut G (Department of Neonatal Medicine Department of Obstetrics and Laboratory of Microbiology Clinique Notre Dame Charleroi Belgium) Group B streptococcal colonization of pregnant women and their neonates epidemiological study and controlled trial of prophylactic treatment of the newborn Acta Paediatr Scand 68 819 1979 —Colonization with group B streptococci of the genital tract was studied in 1 115 women during the last trimester of pregnancy 76 or 6 82% were found to harbour this bacterium The incidence of contamination was significantly higher among Belgian women than among parturients of Mediterranean origin ($p<0\ 001$) It was also more frequent in primigravidae ($p<0\ 05$) and in the poorer ($0\ 10<p>0\ 05$) At the time of admission in the delivery room it was noticed that rupture of the amniotic membranes for more than 24 hours was more often associated with group B streptococcal carriage by the mother ($p<0\ 001$) 29 out of 68 (42 6%) infants born to group B streptococci positive mothers were colonized at birth 67 of them were submitted to a controlled trial of immediate versus delayed penicillin therapy 44 8% and 4? 1% of the neonates were contaminated at birth in each group of treatment respectively No instance of group B streptococcal infection developed in either group This suggests that immediate therapy with penicillin of infants of group B streptococci positive mothers has no definite advantage upon delayed treatment

KEY WORDS Group B streptococcus pregnancy epidemiology antibiotic prophylaxis

Early onset group B streptococcal (GBS) infection represents an important cause of mortality in the neonatal period (1 10 11 17 19) Epidemiological studies have shown that contamination of the neonate is secondary to the presence of GBS in the maternal genital tract (? 3 11 15) Some 2–25% of pregnant women carry this bacterium during the last trimester of pregnancy (2 6 7 11–13 15 16) Some 15–70% of their neonates are found contaminated immediately after birth (2 6 11 13) but only very few of these infants will develop a severe systemic infection (2) the mortality of which is close to 60% (1 10 11 17 19)

Therapeutic trials aiming at prevention of infection in the newborn by eradication of GBS from the maternal organism have been conducted with unconclusive results (11 12 13)

Various approaches to the treatment of infants born to GBS positive mothers have been suggested Steigman (18) raises the question whether a single systematic injection of penicillin G to every neonate after birth could prevent the development of the disease Because early GBS infection often manifests itself through respiratory symptoms Cochran (9) advises penicillin administration to all infants presenting respiratory distress until results of cultures are obtained Ablow et al (1) are in favour of early treatment of newborns presenting factors of risk infants born

Table I. *Correlations between maternal GBS colonization and some factors or events*

Factors	No. of parturiants colonized with GBS	%	p
Origin			
Belgian	62/576	10.76	<0.001
Mediterranean	14/538	2.60	
Gestational status			
Primigravidae	37/414	8.94	<0.05
Plurigravidae	39/701	5.56	
Maternal age			
Less than 20	13/193	6.74	N.S.[a]
21–30	57/790	7.21	
31–35	4/93	4.30	
More than 35	2/39	5.13	
Economic status			
I	25/487	5.13	N.S.
II	8/94	8.51	
III	13/176	7.39	
IV	7/77	9.09	
V	2/15	13.33	
Duration of rupture of membranes			
Less than 24 hours	70/1 090	6.42	<0.001
More than 24 hours	6/25	24.00	

χ square test.
[a] Non significant.

to GBS positive mothers: prematurity, prolonged rupture of the amniotic membranes, early apneic syndrome.

In 1976, two infants born in our Maternity developed a GBS sepsis and one of them died. This prompted the present study. As the epidemiology of GBS contamination during pregnancy in populations living in Belgium is unknown, it was decided to obtain data for the area of Belgium served by our maternity unit. Simultaneously, we tested the hypothesis whether immediate antimicrobial therapy of newborns of GBS positive mothers could prevent the development of a systemic infection.

MATERIAL AND METHODS

Screening during the third trimester of pregnancy

Commencing January 4, 1977, all pregnant women attending the antenatal clinic of our maternity unit had a vaginal culture performed around the 32nd–34th week. Two obstetricians included their private parturients in the study. Only data concerning women who delivered before the end of January 1978 were computed. The maternity unit has an annual delivery rate of about 1700. Half this population is Belgian and the other half are women of Mediterranean origin, many of them being recent immigrants.

Screening in the neonate

Immediately after birth, bacteriological samples were taken from infants born to known GBS positive mothers. Cultures were taken from the external auditory canal, the gastric aspirate and the fetal side of the placenta.

Bacteriological procedures

Whenever feasible, the bacteriological samples were processed immediately. Otherwise they were kept in Stuart transport medium. The swabs or the gastric fluid were spread on 5% agar blood plates under an atmosphere supplemented with CO_2 and inoculated in enriched broth. Identification of the organisms was made by direct examination, Gram staining, study of the hemolytic character of the colonies, resistance to bacitracin and agglutination with commercially available anti sera (Phadebas, Brocades®). No further subtyping was performed.

Controlled study of penicillin therapy of the neonate

Infants born to mothers who had been found GBS carriers were considered for this study. Babies born on an odd day received penicillin G in a dose of 50 000 to 100 000 Units/kg/day intra muscularly in two equal doses from the delivery room on and after collection of the bacteriological samples had been completed. This treatment was continued until the results of the cultures were known. If one site was positive for GBS, the antibiotic was given until the age of 7 days. If the three samples were negative, then the treatment was discontinued. This group is called immediate treatment. Therapy of infants born on an even day was delayed until reception of the results of the cultures. They received penicillin only if at least one sample was positive for GBS. This group is referred as late treatment.

RESULTS

Prenatal screening

1 115 women had a vaginal culture performed during the last trimester of pregnancy. 76 of them (6.82%) carried GBS in the genital tract. Correlations between maternal GBS colonization and some factors or events were analysed (Table 1).

Origin. 62 out of 576 Belgian women (10.7%) were found to carry GBS, whereas 14 out of 538 women of Mediterranean origin (2.6%) were positive, a difference which is highly significant ($p<0.001$, χ square test).

Number of pregnancies 37 out of 414 women (8 9%) who were pregnant for the first time were GBS positive versus 39 out of 701 (5 6%) plurigravidae ($p<0\ 05$)

Maternal age Among 193 women aged less than 20 13 (6 74%) were GBS positive 57 out of 790 (7 21%) 21–30-year old pregnant women were GBS carriers 4 out of 93 (4 3%) were positive in the 31–35 age group and 2 out of 39 (5 13%) women aged more than 35 Although the incidence of GBS colonization falls after the age of 30 the difference does not reach statistical significance

Economic status This was determined on the basis of the husband s profession Five classes were constituted classes I and II were of lower income classes III and IV were middle classes and class V had the higher income The incidence of GBS contamination was the lowest in class I (25/487 or 5 13%) similar in classes II to IV (respectively 8/94 or 8 51% for class II 13/176 or 7 39% for class III and 7/77 or 9 09% for class IV) highest in class V 2/15 or 13 33% A difference of borderline statistical significance ($0\ 10 < p > 0\ 05$) was found between class I and all the others

At time of admission to the delivery room a correlation was established between the duration of rupture of the amniotic membranes and presence of GBS In 1 090 women the membranes were broken for less than 24 hours 70 of them (6 4%) were GBS carriers Among 25 cases of rupture of the membranes for more than 24 hours 6 women (24%) were known GBS carriers This difference is highly significant ($p<0\ 001$)

Contamination of the neonate at birth

68 infants born to the 76 last trimester GBS positive mothers could be studied 29 of them (42 6%) had at least one site positive for GBS A trend to an increase of preterm delivery or low birth weight was observed among infants born to GBS positive mothers 11/107 (10 3%) preterm infants were born of contaminated mothers compared with 65/1008 (6 44%) infants born after 37 weeks 5/43 (11 62%) babies who weighed less than 2 500 g at birth were from GBS positive mothers as opposed to 71/1072 (6 62%) infants of more than 2 500 g However those differences do not reach statistical significance

Controlled study of penicillin therapy of the neonate

Among the 68 infants born to GBS positive mothers one was a stillborn who was found negative for GBS This left 67 babies for the trial 29 were assigned to the immediate treatment group 13 of them (44 8%) were subsequently found colonized with GBS They completed the seven day course of penicillin All of them remained free of infectious symptoms Among the 38 infants of the late treatment group 16 (42 1%) were contaminated and received penicillin once the results of the cultures were known usually at an age of 24 to 48 hours No baby in this group had clinical signs that could be related to GBS infection

No case of systemic GBS disease was observed during the period of the study as compared with two GBS sepsis—one fatal—in 1976

To our knowledge no instance of late GBS meningitis was observed in any patient of either treatment group No secondary ill effect could apparently be attributed to the antibiotic therapy

DISCUSSION

The incidence of 6 8% of colonization with GBS during the last trimester of pregnancy in the population we surveyed is lower than the rate reported by Scandinavian authors 18% in Norway (7) and 14% in Sweden (6) It is close to most data reported from several centres in the USA (2 3 11 15) where however regional variations as wide as a 2 and 25% were evidenced

The population attending our Maternity is heterogeneous as far as its ethnic origin is concerned It was therefore interesting to note

Table I *Correlations between maternal GBS colonization and some factors or events*

Factors	No of parturients colonized with GBS	%	p
Origin			
Belgian	62/576	10 76	<0 001
Mediterranean	14/538	2 60	
Gestational status			
Primigravidae	37/414	8 94	<0 05
Plurigravidae	39/701	5 56	
Maternal age			
Less than 20	13/193	6 74	N S[a]
21–30	57/790	7 21	
31–35	4/93	4 30	
More than 35	2/39	5 13	
Economic status			
I	25/487	5 13	N S
II	8/94	8 51	
III	13/176	7 39	
IV	7/77	9 09	
V	2/15	13 33	
Duration of rupture of membranes			
Less than 24 hours	70/1 090	6 42	<0 001
More than 24 hours	6/25	24 00	

χ square test
[a] Non significant

to GBS positive mothers prematurity prolonged rupture of the amniotic membranes early apneic syndrome

In 1976 two infants born in our Maternity developed a GBS sepsis and one of them died This prompted the present study As the epidemiology of GBS contamination during pregnancy in populations living in Belgium is unknown it was decided to obtain data for the area of Belgium served by our maternity unit Simultaneously we tested the hypothesis whether immediate antimicrobial therapy of newborns of GBS positive mothers could prevent the development of a systemic infection

MATERIAL AND METHODS

Screening during the third trimester of pregnancy

Commencing January 4 1977 all pregnant women attending the antenatal clinic of our maternity unit had a vaginal culture performed around the 32nd–34th week Two obstetricians included their private parturients in the study Only data concerning women who delivered before the end of January 1978 were computed The maternity unit has an annual delivery rate of about 1 /00 Half this population is Belgian and the other half are women of Mediterranean origin many of them being recent immigrants

Screening in the neonate

Immediately after birth bacteriological samples were taken from infants born to known GBS positive mothers Cultures were taken from the external auditory canal the gastric aspirate and the fetal side of the placenta

Bacteriological procedures

Whenever feasible the bacteriological samples were processed immediately Otherwise they were kept Stuart transport medium The swabs or the gastric fl were spread on 5% agar blood plates under an atm phere supplemented with CO_2 and inoculated in enrich broth Identification of the organisms was made by dir examination Gram staining study of the hemolytic ch acter of the colonies resistance to bacitracin and agg tination with commercially available anti sera (Phadeb Brocades®) No further subtyping was performed

Controlled study of penicillin therapy of the neonate

Infants born to mothers who had been found GBS carr were considered for this study Babies born on an odd received penicillin G in a dose of 50 000 to 100 000 Un kg/day intra muscularly in two equal doses from delivery room on and after collection of the bacteriolog samples had been completed This treatment was c tinued until the results of the cultures were known If site was positive for GBS the antibiotic was given u the age of 7 days If the three samples were negative t the treatment was discontinued This group is called mediate treatment Therapy of infants born on an e day was delayed until reception of the results of the tures They received penicillin only if at least one sam was positive for GBS This group is referred as late tre ment

RESULTS

Prenatal screening

1 115 women had a vaginal culture perform during the last trimester of pregnancy 76 them (6 82%) carried GBS in the genital tra Correlations between maternal GBS coloniza tion and some factors or events were analys (Table 1)

Origin 62 out of 576 Belgian women (10 7 were found to carry GBS whereas 14 out 538 women of Mediterranean origin (2 6 were positive a difference which is highly si nificant ($p<0\ 001$ χ square test)

REFERENCES

1 Ablow R C Driscoll S G Effman E L Gross I Jolles C J Uauy R & Warshaw J B A comparison of early onset streptococcal neonatal infection and the respiratory distress syndrome of the new born *N Engl J Med 294* 65 1976
Baker C J & Barrett F F Transmission of group B streptococci among parturient women and their neonates *J Pediat 83* 919 1973
3 Baker C J Barrett F F & Yow M D The influence of advancing gestation on group B streptococcal colonization in pregnant women *Am J Obstet Gynecol 1 2* 8?0 1975
4 Baker C J & Kasper D L Correlation of maternal antibody deficiency with susceptibility to neonatal group B streptococcal infection *N Engl J Med 294* 753 19 6
5 Baker C J Goroff D K Alpert S Crockett V A Zinner S H Evrard J R Rosner B & McCormack W M Vaginal colonization with group B streptococcus A study in college women *J Infect Dis 135* 39 1977
6 Bergqvist G Hurvell B Thal E & Vaclavinkova V Neonatal infections caused by group B streptococci *Scand J Infect Dis 3* ?09 1971
7 Bevanger L Carrier rate of group B streptococci with relevance to neonatal infections *Infection 2* 1?3 1974
8 Bobitt J R The group B beta hemolytic streptococcus *Semin Pe inatal 1* 51 1977
9 Cochran W D Hyaline membrane disease and infection in the newborn *N Engl J Med 294* 844 1976
10 Eickhoff T C Klein J D Daly K Ingall D & Finland M Neonatal sepsis and other infections due to group B beta hemolytic streptococci *N Engl J Med 271* 1? 1 1964
11 Franciosi R A Knostman J D & Zimmerman R A Group B streptococcal neonatal and infant infections *J Pe l atr 8?* 07 1973
1 Gordon J S & Sbarra A J In idence technique of isolation and treatment of group B streptococci in obstetric patients *Am J Obstet Gynecol 126* 10?3 1976
13 Hall R Barnes W Sumathy V Rhodes P & Fayez J Antibiotic treatment of parturient women colonized with group B streptococci *J Pediatr 86* 975 1975
14 Hammerschlag M R Baker C J Alpert S Kasper D L Rosner I Thurston P Webb B J & McCormack W M Colonization with group B s eptococci in girls under 16 years of age *Pediatrics 60* 473 1977
15 Hood M Janney A & Dameron G Beta hemolytic streptococcus group B associated with problems in the perinatal period *Am J Obstet Gyne ol 82* 809 1961
16 Horn K A Zimmerman R A Knostamn J D & Meyer W T Neurological sequelae of group B streptococcal infection *Pediatrics 53* 501 1974
17 Quirante J Ceballos R & Cassidy G Group B beta hemolytic streptococcal infection in the new born *Am J Dis Child 128* 659 1974
18 Steigman A J Bottone E J & Hanna B A Does intramuscular penicillin at delivery prevent group B beta hemolytic streptococcal disease of the newborn infant? *J Pediatr 87* 496 1975
19 Vollman J H Smith W L Ballard E T & Light I J Early onset group B streptococcal disease clinical roentgenographic and pathologic features *J Ped atr 89* 199 1976

Submitted Nov 11 1978
Accepted Feb ?3 1979

(P G) Department of Neonatal Medicine
Clinique Notre Dame
3 Grand Rue
B 6000 Charleroi
Belgium

that the incidence of GBS colonization was significantly higher among Belgian women (10.8%) than in women of Mediterranean origin (2.7%).

A significantly higher incidence was also found in primigravidae women than in pluri-gravidae. Pregnant women from families with a lower economic income had a tendency to be less often contaminated. Maternal age did not seem to play a role.

It is worth stressing that the various factors associated with a lower rate of colonization with GBS were often present together: repeated gestation and low economic status were more frequent in the Mediterranean population.

Factors that could influence the differences in the rate of GBS carriage during pregnancy among the subgroups we studied are not well known. Ethnic or genetic influences are suggested by our findings, although other authors (3, 5) did not demonstrate such differences. Immunological factors such as ability to synthesize antibodies against GBS must also be considered (4, 14). Genital carriage of GBS by male sexual partners is another factor worth investigating (11).

In the present study, we found a correlation between prolonged rupture of the amniotic membranes and previous presence of GBS in the cervicovaginal canal. These results are in accordance with Baker et al. (3). They suggest that presence of GBS could favour premature rupture of the membranes.

Not every infant born to a contaminated mother necessarily acquires this organism during the process of labour and birth. In our series 29 out of 68 (42.6%) were colonized. In other studies, incidences of 71% (2), 23.5% (6), 21.5% (13) and 17.6% (11) were reported. Factors that possibly protect the newborn against contamination by GBS are unknown.

The aim of the study of antimicrobial treatment of infants born to GBS positive mothers was to test the hypothesis that immediate therapy could prevent the development of severe early pneumonic septic forms of GBS disease. When designing the study, it was estimated that the observation of a single case of severe infection in the delayed treatment group would represent an argument in favour of immediate penicillin therapy of these infants. Conversely, the appearance of a septic state in a baby assigned to early treatment would imply that even immediate treatment would be of limited value in prophylaxis of the disease, a statement often suggested by the fulminating aspect of this infection in some babies. As no instance of GBS infection was observed in either group, these questions could not be answered.

Early treatment has several possible inconveniences. It was sometimes difficult for the parents to understand the treatment of an apparently healthy infant. As about 60% of the babies born to GBS positive mothers were not contaminated at birth, systematic treatment would result in unnecessary administration of penicillin to a large number. Penicillin therapy had no apparent untoward effect but could carry a potential risk of subsequent sensitization. Moreover the physiological microbial colonization of the neonate could be modified by this treatment.

Other means of prevention of GBS disease in the newborn are still to be explored. Possible means include the study of protective effects of maternal specific antibodies against some types of GBS (4, 14), new controlled trials of antimicrobial treatment of pregnant women, quantitative estimations of the colonization of the maternal genital tract or invasion of the amniotic cavity at time of incipient labour (8).

Despite the low septic attack rate in neonates but because of the high mortality related to GBS infection, it seems useful to screen pregnant women for presence of this bacterium and to consider their infants as being in great risk of infection if abnormal perinatal events such as prematurity, prolonged rupture of the amniotic membranes, maternal pyrexia, birth asphyxia and early respiratory distress are present.

REFERENCES

1 Ablow R C Driscoll S G Effman E L Gross I Jolles C J Uauy R & Warshaw J B A comparison of early-onset streptococcal neonatal infection and the respiratory-distress syndrome of the newborn N Engl J Med 294 65 1976
2 Baker C J & Barrett F F Transmission of group B streptococci among parturient women and their neonates J Pediatr 83 919 1973
3 Baker C J Barrett F F & Yow M D The influence of advancing gestation on group B streptococcal colonization in pregnant women Am J Obstet Gynecol 122 820 1975
4 Baker C J & Kasper D L Correlation of maternal antibody deficiency with susceptibility to neonatal group B streptococcal infection N Engl J Med 294 753 19 6
5 Baker C J Goroff D K Alpert S Crockett V A Zinner S H Evrard J R Rosner B & McCormack W M Vaginal colonization with group B streptococcus A study in college women J Infect Dis 135 39 1977
6 Bergqvist G Hurvell B Thal E & Vaclavinkova V Neonatal infections caused by group B streptococci Scand J Infect Dis 3 09 1971
7 Bevanger L Carrier rate of group B streptococci with relevance to neonatal infections Infection 2 123 1974
8 Bobitt J R The group B beta hemolytic streptococcus Semin Perinatal 1 51 1977
9 Cochran W D Hyaline membrane disease and infection in the newborn N Engl J Med 294 844 1976
10 Eickhoff T C Klein J D Daly K Ingall D & Finland M Neonatal sepsis and other infections due to group B beta hemolytic streptococci N Engl J Med 271 12 1 1964
11 Franciosi R A Knostman J D & Zimmerman R A Group B streptococcal neonatal and infant infections J Pediatr 82 707 1973
1 Gordon J S & Sbarra A J Incidence technique of isolation and treatment of group B streptococci in obstetric patients Am J Obstet Gynecol 126 1023 1976
13 Hall R Barnes W Sumathy V Rhodes P & Fayez J Antibiotic treatment of parturient women colonized with group B streptococci J Pediatr 86 975 1975
14 Hammerschlag M R Baker C J Alpert S Kasper D L Rosner I Thurston P Webb B J & McCormack W M Colonization with group B s eptococci in girls under 16 years of age Pediatric 60 473 1977
15 Hood M Janney A & Dameron G Beta hemolytic streptococcus group B associated with problems in the perinatal period Am J Obstet Gynecol 82 809 1961
16 Horn K A Zimmerman R A Knostamn J D & Meyer W T Neurological sequelae of group B streptococcal infection Pediatrics 53 501 1974
17 Quirante J Ceballos R & Cassidy G Group B beta hemolytic streptococcal infection in the newborn Am J Dis Child 128 659 1974
18 Steigman A J Bottone E J & Hanna B A Does intramuscular penicillin at delivery prevent group B beta hemolytic streptococcal disease of the newborn infant? J Pediatr 87 496 1975
19 Vollman J H Smith W L Ballard E T & Light I J Early onset group B streptococcal disease clinical roentgenographic and pathologic features J Pediatr 89 199 1976

Submitted Nov 11 1978
Accepted Feb 23 1979

(P G) Department of Neonatal Medicine
Clinique Notre Dame
3 Grand Rue
B-6000 Charleroi
Belgium

Acta Pædiatr Scand 68 825–831 1979

C REACTIVE PROTEIN (CRP) IN EARLY DIAGNOSIS OF NEONATAL SEPTICEMIA

K G SABEL and Ch WADSWORTH

From the Department of Paediatrics I and the Department of Clinical Immunology Institute of Medical Microbiology University of Goteborg Goteborg Sweden

ABSTRACT Sabel K G and Wadsworth Ch (Department of Paediatrics and Department of Clinical Immunology University of Goteborg Sweden) C reactive protein (CRP) in early diagnosis of neonatal septicemia Acta Paediatr Scand 68 825 1979.—The usefulness of CRP in early detection of neonatal septicemia/meningitis and urinary tract infection was studied in a neonatal unit using a semiquantitative latex agglutination as a rapid screening method and electroimmuno assay as reference method for CRP determination In 94% of non infected infants CRP was ≤15 mg/l and 87% had CRP <10 mg/l up to 3 days of age After 3 days of age 96% had CRP <10 mg/l The initial CRP level was increased in 16 out of 18 patients (89%) with bacterial septicemia Low CRP was seen in one patient with total agranulocytosis and septicemia from *Streptococcus* type B and in one patient with *Staphylococcus albus* sepsis A rise in CRP was also seen in very pre term infants with septicemia Increased initial CRP was uncommon in neonatal urinary tract infection (2 of 9) but a rise was seen in 3 additional patients A comparison between CRP total neutrophil blood cell count and band neutrophil count as diagnostic parameters was in favour of CRP at this early stage of infection CRP is of definite value as an aid in early diagnosis of neonatal septicemia and bacterial meningitis

KEY WORDS C reactive protein neutrophils septicemia meningitis urinary tract infection neonates

Septicemia of the neonate is usually a life threatening disease where early diagnosis and prompt treatment are essential for the out come The early signs and symptoms of infection are often unspecific and vague Therefore routine laboratory aids which are simple rapid and specific are badly needed The usefulness of blood parameters such as neutrophil blood cell count (2 3) and band neutrophil count (1) have been reported C reactive protein CRP which is present in only small amounts in serum of healthy individuals (5) is involved in several processes of the unspecific immunologic defence (e g 7 8) In severe infections or inflammatory reactions a striking rise in the serum concentration is often seen Sabel & Hanson (10) have recently shown that during the first five days after diagnosis of septicemia–meningitis in infants increased CRP levels were found in at least 85% of the patients In several cases they observed high CRP values during the first few hours after clinical symptoms had appeared This suggested the possibility that the rise of CRP was sufficiently rapid and specific to serve as a definitive aid in the early diagnosis of septicemia

The present report studies the efficacy of CRP for the early diagnosis of neonatal infections of pre term and term babies and presents a comparison of CRP with total neutrophil and band cell counts in this respect

MATERIAL AND METHODS

A prospective investigation was performed from August 1973 to July 1975 The study included infants up to 30 days of age with suspect general infection at an early stage either because of clinical signs and symptoms or because of chorio-amnioitis or infection of the mother

Of the original 165 infants in the prospective study 36 (22%) had to be excluded because they did not fulfil the criteria (samples for cultivation and CRP taken on different days insufficient samples or samples taken after

Table 1 *Infants with verified septicemia or meningitis Group I a*

CRP=C reactive protein
CSF=Cerebrospinal fluid

Patient no	Age at sampling (days)	CRP (mg/l)	Total neutrophil (count/mm)	Total band (count/mm)	Cultured agent	
					Source	Designation
1	5/24	6	0	0	Blood	*β-Streptococcus* group B
	17/24	60	300	140	Blood CSF	*β Streptococcus* group B
	1½	200	1 700	2 100		
3	17/24	6			Blood	*β-Streptococcus* group B
	2	34				
4	<1	70			Blood CSF	*β Streptococcus* group B
	1	70				
5	1½	200			Blood	*β-Streptococcus* group B
	5	33				
6	3	65	14 200	600	Blood	*β Streptococcus* group B
	6	105				
7	16	9			Blood	*β Streptococcus* group B
	17	[illegible]0				
8		23			Blood	*α Streptococcus*
	3	130				
9	4	1	3 400	150	Blood	*Staphylococcus albus*
10	4	40			Blood	*Staphylococcus albus*
11	7	23	4 700	60	Blood	*Staphylococcus aureus*
	8	2	2 500	90		
12	1/24	74			CSF lungs urine	*Pneumococcus* *Escherichia coli*
13	4	75	3 000	700	Blood CSF	*Escherichia coli*
	7	30				
14	4	0	1 400	250	Blood	*Escherichia coli + enterococcus*
	9	15				
15	5	75	400	930	Blood	*Escherichia coli*
16	7	30			Blood	*Escherichia coli*
	8	140				
17	18	60			Blood	*Escherichia coli*
	3	0				
18	3	100			Blood	*Pseudomonas aeruginosa*
19	4	14			CSF	Coxsackie B virus
	6	15	11 400	9 0		
20	5	6			CSF	ECHO virus type 9
	6					

Died

t 16 mg/l at 0–3 days of age and 8 mg/l at 4–30 days of age for non infected infants

CRP in infants with septicemia or meningitis (Group I a)

This group consisted of 20 infants with verified septicemia or meningitis As is seen in Fig 2 and Table 1 16 out of 18 patients (89%) with bacterial septicemia or meningitis had increased CRP levels in the initial CRP sample Case no 1 with a fatal congenital infection caused by *β Streptococcus* group B had a CRP within the normal range but only one sample was obtained at 5 h of life This patient also had total agranulocytosis One patient (no 9) with *Staph albus* sepsis also had a low

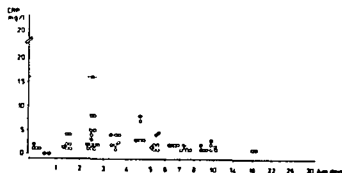


Fig 1 Group III non infected infants (*n*=98) Concentration of CRP in first sample related to age of the infant Normal limit is indicated

antibiotics had been given) From the remaining 129 patients 232 samples were taken for CRP determinations In addition all infants born at the hospital during August 1975 to July 1976 with a diagnosis of septicemia meningitis or urinary tract infection (UTI) were retrospectively studied (55 samples from 12 patients)

Depending on the clinical course and the results of the cultivations the 141 infants were divided into four groups

Group Ia 20 *infected* infants with septicemia or meningitis verified by positive cultures from the blood in the 18 of bacterial origin The other 2 infants had positive viral cultures from the cerebrospinal fluid (CSF) (see Table 1) Four babies had bacterial meningitis with positive cultures from the CSF

Group Ib 9 *infected* infants with urinary tract infection verified by cultivation of *Escherichia coli* (>1000 organisms/ml) in urine obtained by bladder puncture but other cultivations were negative (see Table 2)

Group II 14 *suspect infected* infants where cultivations of blood CSF and bladder urine were negative These patients like those in Groups Ia and b had a clinical course suggesting general infection

Group III 98 *non infected* infants who satisfied the aforementioned criteria for care in the Neonatal Unit but where the cultivations were negative Furthermore the subsequent clinical course was not suggestive of infection and no antibiotic treatment was given

Sampling techniques Urine was obtained by suprapubic bladder puncture and CSF by lumbar puncture Blood for aerobic and anaerobic cultivation was taken from peripheral veins In about one half of the cases capillary blood from free flowing heel pricks was used for the total and differential white cell counts

CRP determinations were performed with two methods on serum from capillary venous or arterial blood often as small a sample as 0 5 ml of blood

Latex agglutination was performed mainly according to the manufacturer s instructions (AG Behringwerke Marburg Germany) using 10 μl aliquots of reagents and patient s serum undiluted and diluted 1/5 and 1/20 with Veronal buffer 75 mmol/l pH 8 2 with 12 3 mmol Ca lactate/l and 6 mmol NaN_3/l After 5 min reaction time the degree of agglutination was read at 12 5 ×s magnification and registered as negative ± 1+ or 2+ An arbitrary scale was established for each batch of latex particles using sera with known CRP content as determined by electroimmuno assay Thus a preliminary semiquantitative report of CRP level as negative <10 10–30 0–40 40–50 >50 mg/l could be received within ? h of sampling

The electroimmuno assay (EIA) originally presented by Laurell (6) was employed for more exact quantitation as described by Wadsworth (11) Plates were run ? 3 times per week with results reported the day after each run

Statistical methods employed were a non parametric tolerance interval (9) and the Wilcoxon rank sum test

RESULTS

CRP in non infected infants (Group III)

The initial CRP values determined with EIA [illegible] the 98 non infected infants during the fi[illegible] month of life are shown in Fig 1 The init[illegible] sample was obtained at 0–3 days of age in [illegible] at 4–7 days in 25 and at 8–30 days in 22 p[illegible] tients CRP values were squewly distribut[illegible] when values for each day were considered [illegible] 0–3 days of age 94% had ≤15 mg/l and 8[illegible] <10 mg/l At 4–7 days of age 96% had le[illegible] than 10 mg/l Using the non parametric tole[illegible] ance interval (95–50 TI) the upper limit was [illegible]

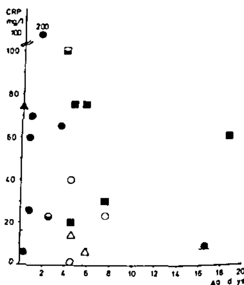


Fig 2 Group Ia infants with verified septicemia/menin[illegible] gitis (*n*=20) Concentration of CRP in first sample relate[illegible] to age Normal limit is indicated Symbols *β-Streptococcus* group B ● *α-Streptococcus* ⊖ *Staph aureus* ○ *Staph albus* ○ *Pneumococcus*+*E coli* ▲ *E coli* ■ *Pseudomonas aeruginosa* ⊟ *Coxsackie B* or ECH[illegible] *meningitis* △

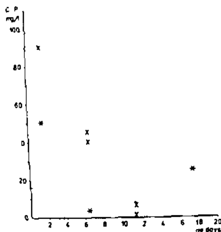


Fig. 4 Group II infants with suspected infection not verified by culture Concentration of CRP in first sample related to age Normal limit is indicated Infants not treated by antibiotics marked by

tation produced moderate to high amounts of CRP When comparing infected pre term babies (27–36 weeks $n=11$) with full term newborns with verified bacterial sepsis/meningitis ($n=7$) no significant difference was found in CRP levels between the groups

CRP versus total neutrophil blood cell count

The results of total neutrophil counts in 71 patients at the time of initial CRP sample was compared with the data presented by Gregory & Hey (2) In the present study an early total neutrophil count fell within the region within which 90% of all normal neutrophil counts are found in 4 out of 8 tested cases of bacterial septicemia/meningitis in all 3 tested cases with UTI and in 5 of the 10 tested cases in Group II Furthermore 20% of the non infected tested babies (Group III) were false positive

CRP versus band neutrophil count

The distribution of early samples of nonsegmented (band) polymorphonuclear leucocytes was investigated in 50 babies during the first 5 days of life With one exception they were all within the 95% range for normal full term infants reported by Akenzua et al (1) Thus 7 infants with bacterial septicemia/meningitis and one case of UTI were rated false negative while 7 out of the 8 tested patients in the suspect infected group were within the normal range

Utility of agglutination results

The agglutination results for the initial samples can be considered in relation to the patient groups With the exception of Group Ib the distribution seems reasonable 85% of the non infected infants had negative ± or + agglutination results while the infected Ia and suspect infected Group II had 10 or 15% with in this range Agglutination findings were initially low in 7 out of 9 infants with verified bacteriuria It should be noted however that of the 23 sera with initial 2+ agglutination which were at first reported as 10–30 mg/l 12 were from infants later designated as non infected Nine of these proved to have EIA ratings in the normal range for age

Some of these 2+ (10–30 mg/l) reports can be followed up in Table 3 where the detailed results are given for second samples with a higher CRP level than that found in the first In most instances the infected infants responded in the next sample taken within 1 or 2 days with high levels of CRP in contrast to the non infected

DISCUSSION

The need is obvious for a quick and safe guide to direct the difficult decision of treatment or no treatment of a neonate with vague symptoms which could be due to sepsis It is of greatest importance for the outcome to catch the earliest possible deviation from normal in any laboratory parameter used for this purpose During the last few years several authors have reported the usefulness of total neutrophil count (2 3 12) or total band neutrophil count (1 12 13) in this respect In the present study neither total neutrophil count nor band neutrophil count were comparable to

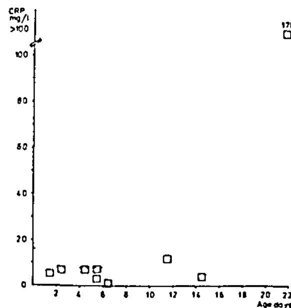


Fig 3 Group Ib infants with verified urinary tract infection (UTI) ($n=9$) Concentration of CRP in first sample related to age Normal limit is indicated UTI from *E coli* □

CRP in his single sample The two cases with viral meningitis had low CRP at 14 and 6 mg/l respectively Four out of 5 cases with verified septicemia or meningitis during the first day of life already had an increased level of CRP The earliest sample was taken at 1 hour of age (case 12)

CRP in infants with bacteriuria (Group Ib)

In the group of babies with bacteriuria 7 of 9 had initial CRP levels within the normal range (Fig 3 Table 2) One patient had a slightly increased CRP of 12 mg/l and another 3 weeks old had an initial CRP of 170 mg/l This patient (no 29) was the only one with symptoms of UTI of several days duration at the day of sampling and the only one with temperature over 38°C The microsedimentation rate was 40 mm/h In subsequent samples from three of four patients with very slight symptoms a marked increase in CRP level was observed In 2 of the 3 patients with increasing CRP no treatment had been given for 3 days after bacteriuria had been ascertained The slight clinical symptoms remained unchanged and the temperature was 37 5 C at its highest

Table 2 *Infants with urinary tract infection verified by cultivation of Escherichia coli from urine taken by bladder puncture Group Ib*
CRP=C reactive protein

Patient no	Age at sampling (days)	CRP (mg/l)
21	1	5
	2	100
	3	32*
22	2	7
	5	100
23	4	7
24	5	7
25	5	3
	9	1
26	6	1
27	11	12
	23	3
28	14	4
	17	60
	20	6
29	21	170
	24	80
	28	5

After treatment had been initiated

CRP in suspect infected infants (Group II)

Ten of the 14 cases of suspected infection not verified with positive culture had high levels of CRP one was slightly increased and 3 had CRP within the normal range (one with aseptic meningitis one with slight aspiration and one with small infiltrates on X ray suggestive of pneumonia) (Fig 4) Seven of the 14 cases had pulmonary infiltrates suggestive of pneumonia or aspirations with secondary pneumonia Two cases with initial CRP 40 and 90 mg/l respectively had symptoms highly suggestive of septicemia but cultures were negative

CRP in relation to gestational age

The initial CRP values taken during the first week of life were grouped according to the gestational age of the infants Four out of 5 infants with septicemia after 27–32 weeks of ges

there is about 15% false negatives in the present series With more experience with the SIA method this figure may be reduced In a newborn with vague symptoms which may or may not indicate bacterial infection CRP as well as the clinical condition should be fol lowed If the clinical condition is satisfactory and the CRP not rising above normal limits antibiotics usually can be withheld In our ex perience the use of CRP as a diagnostic aid has reduced administration of antibiotics in the nursery

ACKNOWLEDGEMENTS

The skilful technical assistance of Miss Lena Beijer and Mrs Bent Bahr is gratefully acknowledged This study was supported by grants from the Medical Faculty Univer sity of Goteborg The Swedish Medical Society s re search fund and the Swedish Medical Research Council (no 15) One of us (Ch W) is grateful for a personal grant from F Hoffmann La Roche Nutley N J USA

REFERENCES

1 Akenzua G I Hui Y T Milner R & Zipursky A Neutrophil and band counts in the diagnosis of neonatal infections Ped atrics 54 38 1974
Gregory J & Hey E Blood neutrophil response to bacterial infection in the first month of life Arch Dis Child 47 747 197
3 Haider S A Polymorphonuclear leucocyte count in diagnosis of infection in the newborn Arch Dis Child 47 394 197
4 Jodal U Lindberg U & Lincoln K Level diagnosis of symptomatic urinary tra t infections in childhood Acta Paediatr Scand 64 701 1975
5 Kindmark C O The concentration of C reactive protein in sera from healthy individuals Scand J Clin Lab Invest 29 407 1972
6 Laurell C B Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies Anal Biochem 15 45 1966
7 Mortensen R F Osmond A P & Gewurz H Ef fects of C reactive protein on the lymphoid system I Binding to thymus-dependent lymphocytes and al teration of their functions J Exper Med 141 821 1975
8 Mortensen R F & Gewurz H Effects of C reactive protein on the lymphoid system II Inhibition of mixed lymphocyte reactivity and generation of cyto toxic lymphocytes J Immunol 116 1244 1976
9 Reed A H Henry R J & Mason W B Influence of statistical methods used on the resulting estimate of normal ranges Clinical Chemistry 17 275 1971
10 Sabel K G & Hanson L Å The clinical useful ness of C reactive protein (CRP) determinations in bacterial meningitis and septi emia in infancy Acta Paediatr Scand 63 381 1974
11 Wadsworth Ch A rapid spot immunoprecipitate assay method applied to quantitating C reactive pro tein (CRP) in pediatric sera Scand J Immunol 6 1 63 1977
12 Xanthou M Leucocyte blood picture in ill newborn babies Arch Dis Child 47 741 1972
13 Zipursky A Palko J Milner R & Akenzua G I The hematology of bacterial infections in premature infants Ped atrics 57 839 1976

Submitted Febr 14 1979
Accepted April 19 1979

(K G S) Department of Pediatrics I
Östra sjukhuset
S-416 85 Goteborg
Sweden

Table 3 *Consecutive samples showing a rise in C reactive protein*
EIA=electroimmuno assay UTI=urinary tract infection

	At least two samples		First sample			Second sample				
			Agglutination			Agglutination				
	n	with rise in second	1/1	1/5	EIA (mg/l)	1/1	1/5	1/20	EIA (mg/l)	Interval (in days)
Gr Ia Infected (*n*=20)	15	7	±	–	9	+	++	±	270	1
			++	–	14	±	–		15	2
			++	–	23	–	++	++	130	1
			++	–	26	++	?	–	34	1
			++	±	30	N D			140	1
			++	++	60	–	++	++	200	1
			–	+	65	N D			105	3
Gr Ib UTI (*n*=9)	5	3	–	–	4	++	+	–	60	3
			++	–	5	+	++	–	100	1
			+	–	7	±	++	–	100	3
Gr II Suspect infected (*n*=14)	13	1	–	–	1	++	+	–	40	<1
Gr III Non infected (*n*=98)	18	3	–	–	1	++	–	–	9	1
			++	–	12	++	–	–	14	1
			++	–	14	±	–		15	2

CRP in the early detection of infection Furthermore there were large numbers of false positives with total neutrophil counts and of false negatives with band neutrophil count

In the present study on CRP we found using a modified standardized semiquantitative latex agglutination that routine results could be obtained within 2 hours From the practical point of view a latex agglutination of – or ± can be considered negative Between 0–3 days of age however some newborns without infection may have values of 15–20 mg/l or 1+ agglutination The range between 10–30 mg/l that is 2+ agglutination is not as clear cut Furthermore samples taken very early in the course of infection may be in this range In doubtful cases it is essential to repeat the sample after a few hours since the rise of CRP seems to be very rapid

A quantitative method which will give the result within a few hours is desirable The electroimmuno assay as performed in our laboratory has proved to be reasonably satisfactory for quantitation although results are not available for 1–2 days and the quantitation may be low for some sera agglutinating at 2+

These challenges may be met with the rapid spot immunoprecipitate assay (SIA) (11)

Cases with neonatal UTI had initial CRP in the normal range In some cases a sharp rise in CRP was seen This may indicate that the infection was first confined to the bladder but later advanced to the kidneys (4)

Infection is most difficult to diagnose in very pre term infants The capacity to form CRP is basal and occurs quite early CRP determination in very pre term infants may prove to be of great value in early detection of infection in these babies

In the neonatal period a CRP rise from causes other than infection seems to be comparatively rare In the present series approximately 5% will have false positive values with a limit set at 15 mg/l at 0–3 days and at 10 mg/l from 4 days on

Thus a high CRP in combination with any symptoms indicating infection is highly suggestive of bacterial infection and should be treated with little risk of overtreatment On the other hand a newborn with symptoms indicating a severe bacterial infection but with negative CRP should also be treated since

Acta Pædiatr Scand 68 833–839 1979

MYCOPLASMA PNEUMONIAE INFECTION

A Retrospective Review of 103 Hospitalised Children

JACQUELINE Y Q MOK I M INGLIS and HAMISH SIMPSON

From the Royal Hospital for Sick Children Edinburgh the Department of Child Life and Health University of Edinburgh and the Regional Virus Laboratory City Hospital Edinburgh Scotland

ABSTRACT Mok J Y Q Inglis J M and Simpson H (The Royal Hospital for Sick Children Edinburgh Department of Child Life and Health University of Edinburgh and Regional Virus Laboratory Edinburgh Scotland) *Mycoplasma pneumoniae* Infection—A Retrospective Review of 103 Hospitalised Children Acta Paediatr Scand 68 833 1979—The clinical aspects of *Mycoplasma pneumoniae* infection in 103 children under 12 years admitted to hospital over an eight year period were reviewed retrospectively Respiratory illnesses occurred in 87 (85%) cases The prevalence of lower respiratory tract involvement was similar in both pre-school and school children Cough was the commonest symptom at all ages Coryzal symptoms and wheeze were common in pre school children Most infants had signs of pharyngitis or otitis media Non specific symptoms—fever lethargy malaise anorexia and vomiting—were common accompaniments in children older than one year of age Non respiratory illnesses in 16 (15%) patients included gastroenteritis convulsions non specific skin rashes and limb pains The duration of stay in hospital ranged from two to 30 days (median five days) with apparent clinical recovery and resolution of chest X ray abnormalities within three months in 78 (76%) patients seen for review

KEY WORDS *Mycoplasma pneumoniae* infection hospitalised children

The spectrum of disease caused by *Mycoplasma pneumoniae* is well documented (6 16) Reports in children have been concerned mainly with out patients in day care centres (7) and residents in children s homes (2 10) Infections are often mild and many children remain symptom free There have been few reports of series of children admitted to hospital (3 21) In a report of 44 such children during an 18 month epidemic period Stevens et al (21) emphasised the importance of lower respiratory tract involvement and serious non respiratory manifestations of infection

Little attention has been paid to the effect of age on clinical presentation We report here a retrospective hospital based study of 103 children under the age of 12 with *M pneumoniae* infection with particular reference to the clinical features in three different age groups—under one year one to five years and five to 12 years

MATERIALS AND METHODS

The names of all children admitted to the Royal Hospital for Sick Children in Edinburgh with *M pneumoniae* infection during the period from January 1968 to December 1975 were obtained from the records of the Regional Virus Laboratory in Edinburgh Their case records were then traced and analysed Initially laboratory investigations had been carried out on the basis of clinical suspicion of *M pneumoniae* infection However from 1972 onwards over 70% of patients admitted to hospital with respiratory tract infections were studied in conjunction with routine virological investigations The series may therefore be biased to include a high proportion of patients with respiratory illnesses No attempt has been made to assess the contribution of *M pneumoniae* infection to the overall pattern of respiratory infections in hospital

Throat and nasal swabs obtained from each child within a day of admission were placed in the same bottle of transport medium (3 ml Hanks balanced salt solution containing 0 2% bovine albumin ampicillin 200 μg/ml and penicillin 150 I U /ml) and transported to the laboratory within one hour Before inoculation within the following three hours swabs were shaken (to liberate the cells from the cotton pledgets) and 0 25 ml added to a selective medium for *M pneumoniae* (5) Cultures were incubated at 36 C for at least two months Sera were tested for complement fixation antibodies to *M pneumoniae* (4)

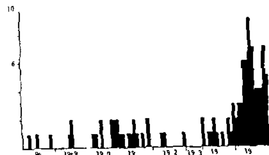


Fig 1 Distribution of admissions of children with M pneumoniae infection by month and year

Table 3 *White cell count (grouped) in relation to age group in 102 children with M pneumonia infection*

Range	Total white count (/mm³)	Age group (years) 0–1	1–5	5	Total number
Normal	4 000–10 000	6	16	27	49
High	>10 000	8	22	22	57
Low	< 4 000	1			1

received two or more drugs Of the antibiotics used ampicillin was the most commonly prescribed (57%) followed by penicillin (34%) The remaining 9% received cotrimoxazole sulphonamides tetracyclines cephalosporins or erythromycin

Eighty seven patients (85%) had respiratory illnesses The main respiratory symptoms were nasal discharge cough and wheeze with cough the predominant symptom at all ages Coryzal symptoms wheeze and vomiting were commonest under the age of five whereas fever malaise lethargy anorexia and headache were least common in this age group The duration of symptoms prior to admission was less than one week in 47 patients (46%) one to two weeks in 24 (24%) two to four weeks in 14 (14%) and over a month in 16 (16%)

Fig 3 gives the main physical signs elicited on examination Signs of inflammation in the upper respiratory tract indicative of rhinitis pharyngitis and otitis media affected a high proportion of patients in the first year of life After the age of one year crepitations in the chest was the most consistent sign of lower respiratory tract involvement Sixteen patients (15%) had no respiratory symptoms Their

Table 2 *Uncommon symptoms and signs in children with M pneumoniae infection*

Symptom/sign	No of patients
Chest pain	7
Weight loss	7
Muscle and joint pains	7
Stridor	6
Mucocutaneous lesions	6
Diarrhoea	4
Meningism	4
Drowsiness	4
Haemoptysis	[illegible]
Haematuria	1

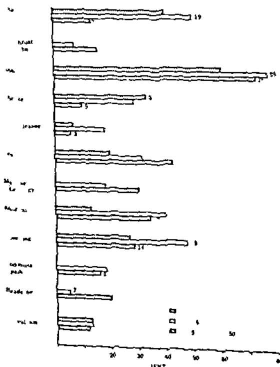


Fig 2 Symptoms by age of 103 children hospitalised for M pneumoniae infection

Table 1 *Diagnostic criteria of M pneumoniae infections in 103 children*

Age group (years)	Number of patients with maximum CF antibody titre to *M pneumoniae*							Number of patients with significant changes *in titre*		*M pneumoniae* isolated
	≥160	320	640	256	512	1 024	Total	Rise	Fall	
<1	1	–	–	5	–	–	6	5	–	3
1–5	–	2	1	20 (2)	1	2	26 (2)	7 (1)	1	10 (3)
>5	4	2	9	17 (3)	3	2 (1)	37 (4)	8 (2)	1	8 (6)
Total	5	4	10	42 (5)	4	4 (1)	69 (6)	20 (3)	2	21 (9)[a]

No of patients with positive isolates in parenthesis
[a] No of patients with antibody measurements in parenthesis

Table 1 summarises the laboratory criteria for diagnosing *M pneumoniae* infection This was based on positive cultures for *M pneumoniae* in 21 patients (20%) a four fold rise or fall in antibody titre in 19 (18%) and a single high titre (≥160 or ≥256) in 63 (62%) The method of reporting antibody titres was changed during the third year of the study

It had been routine clinical practice for several years to investigate single serum samples from children with acute respiratory illnesses admitted to one of three medical wards and to obtain second samples if symptoms had not resolved in the subsequent three to four weeks We have therefore analysed the results obtained in single serum samples from 100 consecutive cases admitted during the first six months in 1976 (the year following an epidemic) Table 1*a* gives the results Ninety six patients had antibody titres of 64 or less

RESULTS

Demographic

There were 62 males and 41 females—a male female ratio of 1 5 1 Their ages ranged from 0 1 to 11 2 years (mean 5 1) Fifteen patients were less than one year old (14%) 38 between one and five years (37%) and 50 over the age of five (49%)

Fig 1 shows the number of cases admitted each year during the study period Most were admitted during an epidemic in 1975

Most children had been healthy prior to the index illness Sixteen (15%) were known to suffer from asthma Ten (10%) had pre existing conditions—congenital heart disease (2) Down s syndrome (2) leukaemia in remission (2) spastic diplegia (1) epilepsy (1) multiple congenital abnormalities (1) and laryngeal papillomata (1)

Blood samples were taken during the acute phase of illness at the time of admission to hospital The duration of illness prior to admission was similar in patients with single low antibody titres and those in whom isolates were obtained The length of stay in hospital ranged from two to thirty days (median five days) In nine (9%) patients at least one other family member had similar symptoms

Clinical

Fig 2 shows the main symptoms according to age group Fever malaise lethargy and anorexia were the most common general symptoms Gastrointestinal complaints (vomiting and/or abdominal pain) were found in 42 patients (41%) while headaches and/or convulsions affected 24 (23%) Six children presented with generalised convulsions

Sixty five patients (63%) had been treated with antibiotics prior to admission and 15 had

Table 1*a* *Antibody titres to M pneumoniae in single serum specimens from 100 consecutive children admitted to hospital with acute respiratory illnesses in 1976*

	Titre					
	<16	16	32	64	128	256
Number	34	21	24	17	0	4

Table 4 *Percentage of patients with high normal or low absolute neutrophils and lymphocyte counts derived from absolute differential white cell counts in 102 children with M pneumoniae infection*

Type of white cell	High	Normal	Low
Neutrophils	39	59	2
Lymphocytes	1	69	30

lated in 64 (74%) *Staphylococcus aureus* (nine patients) *Haemophilus influenzae* (8) group A betahaemolytic streptococci (5) and pneumococci (1) were the bacteria isolated In sputum samples from 18 patients *H influenzae* (seven patients) group A betahaemolytic streptococci (1) and *Pseudomonas pyocyaneus* (1) were isolated The proportion of positive isolates from nose and throat swabs or sputum was higher in patients who had not received antibiotics This was not significant statistically

Cerebrospinal fluid was examined in 17 patients (16%) suspected of having meningitis The lymphocyte count was increased in five and in two samples ECHO virus type 9 was isolated Of two patients with an increase in neutrophils one had meningococcal meningitis Cerebrospinal fluid was sterile in the remaining patients

Two five year old children had co-existing infections outwith the respiratory tract One presented with headache vomiting and abdominal pain and was also found to have a urinary tract infection The other who gave a two-week history of fever anorexia and vomiting also had malaria

Treatment and progress

Patients with radiological evidence of pneumonia were treated with chest physiotherapy and humidified oxygen where indicated on clinical grounds Eighty patients (78%) were treated with antibiotics Ampicillin (40%) and penicillin (29%) were the antibiotics most commonly prescribed Drugs considered to be effective against *M pneumoniae* (erythromycin or tetracycline) were first choice antibiotics in only six patients (6%) A further 25 (24%) were treated with these antibiotics when laboratory results became available

Thirty seven patients (36%) had a temperature exceeding 38°C on admission which in most cases became normal within two days

Following discharge 78 patients (76%) were seen for review at intervals varying from one week to three months In 61 (78%) the chest X ray had returned to normal a month after the initial illness At two months only three patients had residual abnormalities which had cleared a month later

DISCUSSION

This report describes the clinical features of *Mycoplasma pneumoniae* infection in children ill enough to require admission to hospital The question arises whether single low antibody titres (160 or 256) are reliable (Table 1) in the diagnosis of recent *M pneumoniae* infections Our data does not allow a complete answer as we have not measured antibody titres in symptom free children However many children with acute respiratory illnesses had low antibody titres in the acute phase of illness (Table 1a) even in the year following an epidemic of *M pneumoniae* infection In addition we are unaware of amnestic reactions in relation to previous *M pneumoniae* infections It seems unlikely that significant error is introduced by imprecise criteria of diagnosis

More than half of our patients were of pre school age and almost one sixth less than a year old This may seem surprising as it has been stated that pre school children usually have mild or asymptomatic infection (7) The fact that very young or ill children are most likely to be admitted to hospital may explain the age bias in the present series

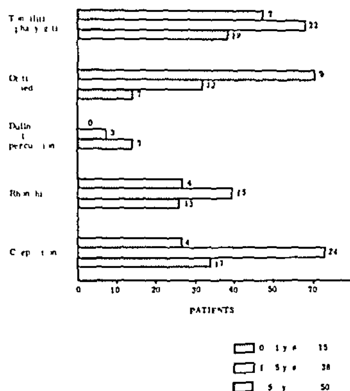


Fig 3 Clinical signs by age of 103 children hospitalised for *M pneumoniae* infection

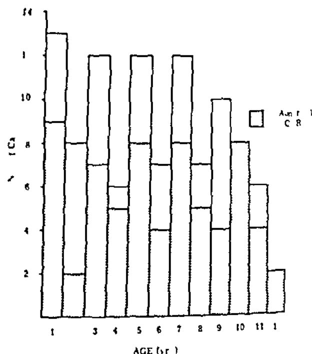


Fig 4 Proportion of children by age with radiological abnormalities

presenting complaints were gastro intestinal (7) convulsions (5) skin rashes (2) limb pains (1) and fever (1)

Table 2 lists uncommon symptoms and signs found in 37 of our 103 patients

Laboratory investigations

Fig 4 shows the proportion of children by age with radiological abnormalities Chest X rays were abnormal in 66 of 94 patients (70%) including peribronchial infiltrates (48%) consolidation of one or more lobes (42%) and overinflation (10%) When present infiltrates were bilateral and diffuse (43% of the X rays showing infiltrates) Of the films with pneumonic consolidation 61% showed the lower lobes to be affected Pleural effusion was noted in only one X ray

Haemoglobin ranged from 9 7 to 15 2 g/dl (mean 12 5) Tables 3 and 4 show the results of total and differential white cell counts performed in 102 children in relation to normal values (1 17 18) Erythrocyte sedimentation rate (ESR Westergren method) in 97 patients was normal in 30 (31%) raised in 62 (64%) and equivocal in 5 (5%) Raised ESRs ranged from 12 to 114 mm/h (mean 42)

Sweat electrolyte concentrations were normal in children with a history of recurrent lower respiratory tract illnesses

Blood films in 54 patients showed rouleaux formation in 31 (57%) atypical lymphocytes in 21 (39%) and toxic granulation of neutrophils in 19 (35%) Some patients had more than one abnormal finding on the blood film

Viruses were isolated or viral infection inferred serologically in 19 patients These included para influenza virus type 1 (four patients) para influenza virus type 3 (1) adenovirus type 2 (3) influenza A virus (2) Coxsackie virus B3 (2) Coxsackie virus B4 (1) ECHO virus type 1 (1) ECHO virus type 9 (2) Herpes simplex virus (2) and measles virus (1)

Bacteriological studies were carried out in nasal and throat swabs from 87 patients 56 (64%) of whom had been treated with antibiotics Commensal organisms only were iso

ACKNOWLEDGEMENT

We thank Mrs D Tervit for secretarial assistance

REFERENCES

1 Altman P L & Dittmer D S *Blood and other body fluids* Federation of American Societies for Experimental Biology Washington D C 1961
Baernstein H D Trevisani E Axtell S & Quilligan J J *Mycoplasma pneumoniae* (Eaton atypical pneumonia agent) in children s respiratory infections *J Pediatr 66* 8 9 1965
3 Bain H H Stewart S & Simpson H Infections due to *Mycoplasma pneumoniae* in children *Arch Dis Child 46* 739 1971
4 Bradstreet S M P & Taylor C E D Technique of omplement fixation test applicable to the diagnosis of viral disease *Monthly Bulletin of Ministry of Health and Public Health Laboratory Service 21* 96 196?
5 Chanock R M Fox H H James W D Cuterkunst R R White R J & Senterfit L B Epidemiology of *Mycoplasma pneumoniae* infection in military recruits *Ann NY Acad Sci 143* 484 1967
6 Denny F W Clyde W A & Glezen W P *Mycoplasma pneumoniae* disease Clinical spectrum pathophysiology epidemiology and control *J Infect Dis 123* 74 1971
7 Fernald G W Collier A M & Clyde W A Jr Respiratory infections due to *Mycoplasma pneumoniae* in infants and children *Paediatrics 55* 3?7 1975
8 Foy H M & Alexander E R *Mycoplasma pneumoniae* infections in childhood *In* I Schulman (ed) *Advances in Pediatrics 16* 301 Book Medical Publishers 1969
9 Foy H M Loop J Clarke E R Mansy A W Spence W F Feigl P & Grayston J T Radiographic study of *Mycoplasma pneumoniae* pneumonia *Am Rev Resp Dis 108* 469 1973
10 Glezen W P Thornburg G Chin T Y D & Wenner H A Significance of Mycoplasma infections in children with respiratory disease *Pediatrics 39* 516 1967
11 Hers J F P & Masurel N Infections with *Mycoplasma pneumoniae* in civilians in the Netherlands *Ann NY Acad Sci 143* 447 1967
1 Hodges G R Fass R G & Saslaw S Central nervous system disease associated with *Mycoplasma pneumoniae* infection *Arch Intern Med 130* 277 197?
13 Kjellman B Pulmonary function in children with *Mycoplasma Pneumoniae* pneumonia *Infection 4* Suppl *1* 71 1976
14 Lerer R J & Kalavsky S M Central nervous system disease associated with *Mycoplasma pneumoniae* infection Report of five cases and review of literature *Pediatrics 52* 658 1973
15 Iok J Y Q Waugh P R & Simpson H *Mycoplasma pneumoniae* infection—A follow up study of 50 children with respiratory illnesses Accepted for publication by *Arch Dis Child* November 1978
16 Murray H W Masur H Senterfit L B & Roberts R B The protean manifestations of *Mycoplasma pneumoniae* infection in adults *Am J Med 5? ?4?* 1975
17 Osgood E E Baker R L Brownlee I E Osgood M W Ellis D M & Cohen W Total differential and absolute leukocyte counts and sedimentation rates of children four to seven years of age *Am J Dis Child* 58 61 1939
18 Osgood E E Baker R L Brownlee I E Osgood M W Ellis D M & Cohen W Total differential and absolute leukocyte counts and sedimentation rates for healthy children Standards for children eight to fourteen years of age *Am J Dis Child 58* ?82 1939
19 Rasch J R & Mogabgab W J Therapeutic effect of erythromycin on *Mycoplasma pneumoniae* pneumonia *Antimicrob Agents Chemother 1965* 693 1966
?0 Riski H & Alanko K Mycoplasma and influenze pneumonia in a series of 214 adults *Scand J Resp Dis* Suppl 89 235 1974
21 Stevens D Swift P G F Johnston P G B Kearney P J Corner B D & Burman D *Mycoplasma pneumoniae* infections in children *Arch Dis Child 53* 38 1978

Submitted De 18 1978
Accepted April 4 1979

(J Y Q M) Department of Child Life and Health
University of Edinburgh
17 Hatton Place
Edinburgh EH9 1UW
Scotland Great Britain

Fever chills cough headache general malaise and lethargy are prominent symptoms in both adults and older children with *M pneumoniae* infection (16) where as gastro intestinal and musculo skeletal symptoms though common are usually minor Gastro intestinal symptoms occurred in 42% of all cases and were often severe enough to dominate the clinical picture In patients under the age of five cough wheeze and vomiting were the most frequent specific symptoms

At all ages clinical signs are of limited diagnostic value A previous study of children in a day care centre (7) suggested that lower respiratory tract involvement is rare in pre school children The high proportion of pre school children with lower respiratory infection and radiological abnormalities in our series may be partly explained by the fact that few children in this age group are admitted to hospital solely on the basis of upper respiratory tract infection Similar findings have been reported in a comparable hospital based series (21)

In a review of central nervous system manifestations of *M pneumoniae* infections Hodges et al (12) found that in adults aseptic meningitis meningo encephalitis psychosis peripheral neuropathy cerebellar ataxia transverse myelitis and hemiplegia may be associated with respiratory disease Cranial nerve mononeuropathy has also been reported (16) Similar findings have been described in children (14) where it was noted that patients under the age of 14 tend to be more severely affected than older patients though residual abnormalities were usually mild Neurological symptoms or signs were present in 24 (23%) of our patients and occurred throughout childhood Cerebrospinal fluid findings were consistent with previous reports (12 14) No attempt was made to detect antibodies or culture *M pneumoniae* in cerebrospinal fluid

Mucocutaneous musculo skeletal and haematological complications of *M pneumoniae* infection although not uncommon in children (8) were seldom seen in the present series

Like clinical signs chest X rays and haematological investigations were of limited help in confirming the diagnosis Foy et al (9) suggested that punctate mottling on chest X rays implying segmental or patchy consolidation and central dense infiltrates were helpful diagnostic clues albeit not pathognomonic In both adults (20) and children (21) the leucocyte count is normal or increased with the neutrophils showing a shift to the left We observed a similar trend but noted a depressed absolute lymphocyte count in 30% of our patients An increase in the erythrocyte sedimentation rate in many of our patients accords with previous experience (20 21)

Mixed infection or bacterial superinfection associated with *M pneumoniae* is said to be uncommon (6 11) Nineteen (19%) of our patients also had viral infections but no associations with a specific viral agent was encountered

Lack of response to antibiotic therapy usually with ampicillin or penicillin was one of the main indications for hospital referral Even so most patients were treated with these antibiotics in hospital This suggests that hospital staff did not believe that antibiotic therapy had been adequate or that they did not consider the possibility of *M pneumoniae* infection Erythromycin and tetracycline have been held to be effective in treatment (19) yet only six patients were treated primarily with these antibiotics either by their family doctor or in hospital

Illnesses caused by *M pneumoniae* vary from asymptomatic to severe though there have been no reports of fatalities in children Clinical recovery appears to be complete In our short term follow up of patients with pulmonary involvement chest X rays had invariably returned to normal within three months It has been shown previously that functional recovery may be delayed (13) Our long term follow up findings (15) confirm that functional abnormalities may be present some years following apparent recovery suggesting the possibility of residual lung damage

ACKNOWLEDGEMENT

We thank Mrs D Tervit for secretarial assistance

REFERENCES

1 Altman P L & Dittmer D S *Blood and other body fluids* Federation of American Societies for Experimental Biology Washington D C 1961
2 Baernstein H D Trevisani E Axtell S & Quilligan J J *Mycoplasma pneumoniae* (Eaton atypical pneumonia agent) in children s respiratory infections *J Pediatr* 66 829 1965
3 Bain H H Stewart S & Simpson H Infections due to *Mycoplasma pneumoniae* in children *Arch Dis Child* 46 739 1971
4 Bradstreet S M P & Taylor C E D Technique of complement fixation test applicable to the diagnosis of viral disease *Monthly Bulletin of Ministry of Health and Public Health Laboratory Service* 21 96 196
5 Chanock R M Fox H H James W D Cuterkunst R R White R J & Senterfit L B Epidemiology of *Mycoplasma pneumoniae* infection in military recruits *Ann NY Acad Sci* 143 484 1967
6 Denny F W Clyde W A & Glezen W P *Mycoplasma pneumoniae* disease Clinical spectrum pathophysiology epidemiology and control *J Infect Dis* 123 74 1971
7 Fernald G W Collier A M & Clyde W A Jr Respiratory infections due to *Mycoplasma pneumoniae* in infants and children *Paediatrics* 55 327 1975
8 Foy H M & Alexander E R *Mycoplasma pneumoniae* infections in childhood *In* I Schulman (ed) *Advances in Pediatrics* 16 301 Book Medical Publishers 1969
9 Foy H M Loop J Clarke E R Mansy A W Spence W F Feigl P & Grayston J T Radiographic study of *Mycoplasma pneumoniae* pneumonia *Am Rev Resp Dis* 108 469 1973
10 Glezen W P Thornburg G Chin T Y D & Wenner H A Significance of Mycoplasma infections in children with respiratory disease *Pediatrics* 39 516 1967
11 Hers J F P & Masurel N Infections with *Mycoplasma pneumoniae* in civilians in the Netherlands *Ann NY Acad Sci* 143 447 1967
12 Hodges G R Fass R G & Saslaw S Central nervous system disease associated with *Mycoplasma pneumoniae* infection *Arch Intern Med* 130 277 1972
13 Kjellman B Pulmonary function in children with *Mycoplasma Pneumoniae* pneumonia *Infection* 4 Suppl 1 71 1976
14 Lerer R J & Kalavsky S M Central nervous system disease associated with *Mycoplasma pneumoniae* infection Report of five cases and review of literature *Pediatrics* 52 658 1973
15 Lok J Y Q Waugh P R & Simpson H *Mycoplasma pneumoniae* infection—A follow up study of 50 children with respiratory illnesses Accepted for publication by *Arch Dis Child* November 1978
16 Murray H W Masur H Senterfit L B & Roberts R B The protean manifestations of *Mycoplasma pneumoniae* infection in adults *Am J Med* 52 742 1975
17 Osgood E E Baker R L Brownlee I E Osgood M W Ellis D M & Cohen W Total differential and absolute leukocyte counts and sedimentation rates of children four to seven years of age *Am J Dis Child* 58 61 1939
18 Osgood E E Baker R L Brownlee I E Osgood M W Ellis D M & Cohen W Total differential and absolute leukocyte counts and sedimentation rates for healthy children Standards for children eight to fourteen years of age *Am J Dis Child* 58 282 1939
19 Rasch J R & Mogabgab W J Therapeutic effect of erythromycin on *Mycoplasma pneumoniae* pneumonia *Antimicrob Agents Chemother* 1965 693 1966
20 Riski H & Alanko K Mycoplasma and influenze pneumonia in a series of 214 adults *Scand J Resp Dis* Suppl 89 235 1974
21 Stevens D Swift P G F Johnston P G B Kearney P J Corner B D & Burman D *Mycoplasma pneumoniae* infections in children *Arch Dis Child* 53 38 1978

Submitted Dec 18 1978
Accepted April 4 1979

(J Y Q M) *Department of Child Life and Health*
University of Edinburgh
17 Hatton Place
Edinburgh EH9 1UW
Scotland Great Britain

Fever chills cough headache general malaise and lethargy are prominent symptoms in both adults and older children with *M pneumoniae* infection (16) whereas gastro intestinal and musculo skeletal symptoms though common are usually minor Gastro intestinal symptoms occurred in 42% of all cases and were often severe enough to dominate the clinical picture In patients under the age of five cough wheeze and vomiting were the most frequent specific symptoms

At all ages clinical signs are of limited diagnostic value A previous study of children in a day care centre (7) suggested that lower respiratory tract involvement is rare in pre school children The high proportion of pre school children with lower respiratory infection and radiological abnormalities in our series may be partly explained by the fact that few children in this age group are admitted to hospital solely on the basis of upper respiratory tract infection Similar findings have been reported in a comparable hospital based series (21)

In a review of central nervous system manifestations of *M pneumoniae* infections Hodges et al (12) found that in adults aseptic meningitis meningo encephalitis psychosis peripheral neuropathy cerebellar ataxia transverse myelitis and hemiplegia may be associated with respiratory disease Cranial nerve mononeuropathy has also been reported (16) Similar findings have been described in children (14) where it was noted that patients under the age of 14 tend to be more severely affected than older patients though residual abnormalities were usually mild Neurological symptoms or signs were present in 24 (23%) of our patients and occurred throughout childhood Cerebrospinal fluid findings were consistent with previous reports (12 14) No attempt was made to detect antibodies or culture *M pneumoniae* in cerebro spinal fluid

Mucocutaneous musculo skeletal and haematological complications of *M pneumoniae* infection although not uncommon in children (8) were seldom seen in the present series

Like clinical signs chest X rays and haema tological investigations were of limited hel in confirming the diagnosis Foy et al (9) sug gested that punctate mottling on chest X rays implying segmental or patchy consolidatior and central dense infiltrates were helpful diag nostic clues albeit not pathognomonic I both adults (20) and children (21) the leucocyt count is normal or increased with the neutro phils showing a shift to the left We observed similar trend but noted a depressed absolut lymphocyte count in 30% of our patients A increase in the erythrocyte sedimentation rat in many of our patients accords with previou experience (20 21)

Mixed infection or bacterial superinfectio associated with *M pneumoniae* is said to b uncommon (6 11) Nineteen (19%) of ou patients also had viral infections but no asso ciations with a specific viral agent was en countered

Lack of response to antibiotic therapy usually with ampicillin or penicillin was on of the main indications for hospital referra Even so most patients were treated with thes antibiotics in hospital This suggests that hos pital staff did not believe that antibiotic the rapy had been adequate or that they did nc consider the possibility of *M pneumonia* infection Erythromycin and tetracycline hav been held to be effective in treatment (19) ye only six patients were treated primarily wit these antibiotics either by their family docto or in hospital

Illnesses caused by *M pneumoniae* vary from asymptomatic to severe though there have been no reports of fatalities in children Clinical recovery appears to be complete I our short term follow up of patients with pul monary involvement chest X rays had in variably returned to normal within three months It has been shown previously that functional recovery may be delayed (13) Our long term follow up findings (15) confirm that functional abnormalities may be present some years following apparent recovery suggesting the possibility of residual lung damage

ACKNOWLEDGEMENT

We thank Mr D Tervit for secretarial assistance

REFERENCES

1 Altman P L & Dittmer D S *Blood and other body fluids* Federation of American Societies for Experimental Biology Washington D C 1961
Baernstein H D Trevisani E Axtell S & Quilligan J J *Mycoplasma pneumoniae* (Eaton atypical pneumonia agent) in children s respiratory infections *J Ped tr* 66 8?3 1965
3 Bain H H Stewart S & Simpson H Infections due to *Mycoplasma pneumoniae* in children *Arch Dis Child* 46 739 1971
4 Bradstreet S M P & Taylor C E D Technique of complement fixation test applicable to the diagnosis of viral disease *Monthly Bulletin of Ministry of Health and Public Health Laboratory Service* 21 96 196?
5 Chanock R M Fox H H James W D Cuterkunst R R White R J & Senterfit L B Epidemiology of *Mycoplasma pneumoniae* infection in military recruits *Ann NY Acad Sci* *143* 484 1967
6 Denny F W Clyde W A & Glezen W P *Mycoplasma pneumoniae* disease Clinical spectrum pathophysiology epidemiology and control *J Infect Dis* *123* 74 1971
7 Fernald G W Collier A M & Clyde W A Jr Respiratory infections due to *Mycoplasma pneumoniae* in infants and children *Paediatrics* *55* 3 7 1975
8 Foy H M & Alexander E R *Mycoplasma pneumoniae* infections in childhood *In* I Schulman (ed) *Advances in Pediat ics* 16 301 Book Medical Publishers 1969
9 Foy H M Loop J Clarke E R Mansy A W Spence W F Feigl P & Grayston J T Radiographic study of *Mycoplasma pneumoniae* pneumonia *Am Rev Resp D s* *108* 469 1973
10 Glezen W P Thornburg G Chin T Y D & Wenner H A Significance of Mycoplasma infections in children with respiratory disease *Pediatrics* 39 516 1967
11 Hers J F P & Masurel N Infections with *Mycoplasma pneumon ae in ci ilians in the Netherlands* *Ann NY Acad Sci* *143* 447 1967
1 Hodges G R Fass R G & Saslaw S Central nervous system disease associated with *Mycoplasma pneumoniae* infection *Arch Intern Med* *130* 277 1972
13 Kjellman B Pulmonary function in children with *Mycoplasma Pneumoniae* pneumonia *Infection 4 Suppl 1* 71 1976
14 Lerer R J & Kalavsky S M Central nervous system disease associated with *Mycoplasma pneumoniae* infection Report of five cases and review of literature *Pediatrics 5?* 658 1973
15 Lok I Y Q Waugh P R & Simpson H *Mycoplasma pneumoniae* infection—A follow up study of 50 children with respiratory illnesses Accepted for publication by *Arch Dis Child* November 1978
16 Murray H W Masur H Senterfit L B & Roberts R B The protean manifestations of *Mycoplasma pneumoniae* infection in adults *Am J Med* 5? 24? 1975
17 Osgood E E Baker R L Brownlee I F Osgood M W Ellis D M & Cohen W Total differential and absolute leukocyte counts and sedimentation rates of children four to seven years of age *Am J Dis Child* *58* 61 1939
18 Osgood E E Baker R L Brownlee I E Osgood M W Ellis D M & Cohen W Total differential and absolute leukocyte counts and sedimentation rates for healthy children Standards for children eight to fourteen years of age *Am J Dis Child 58* 282 1939
19 Rasch J R & Mogabgab W J Therapeutic effect of erythromycin on *Mycoplasma pneumoniae* pneumonia *Antimicrob Agents Chemothe 1965* 69? 1966
?0 Riski H & Alanko K Mycoplasma and influenze pneumonia in a series of 214 adults *Scand J Resp Dis* Suppl 89 235 1974
?1 Stevens D Swift P G F Johnston P G B Kearney P J Corner B D & Burman D *Mycoplasma pneumon ae* infections in children *Arch Dis Child 53* 38 1978

Submitted Dec 18 1978
Accepted April 4 1979

(J Y Q M) Department of Child Life and Health
University of Edinburgh
17 Hatton Place
Edinburgh EH9 1UW
Scotland Great Britain

Acta Pædiatr Scand 68 841–845 1979

T AND B LYMPHOCYTE SUBPOPULATIONS AND LEUKOCYTE TERMINAL DEOXYNUCLEOTIDYL TRANSFERASE IN ENERGY PROTEIN UNDERNUTRITION

R K CHANDRA

From the Department of Pediatrics Faculty of Medicine Memorial University of Newfoundland St John s Newfoundland Canada

ABSTRACT Chandra R K (Department of Pediatrics Faculty of Medicine Memorial University of Newfoundland St John s Newfoundland Canada) T and B lymphocyte subpopulations and leukocyte terminal deoxynucleotidyltransferase in energy protein undernutrition Acta Paediatr Scand 68 841 1979.—Children with energy protein undernutrition showed a reduction in the number of circulating T lymphocytes identified on the basis of their ability to form rosettes with sheep red blood cells T cells with a receptor for IgM (T_μ) were decreased whereas T cells with a receptor for IgG (T_γ) were increased Surface immunoglobulin bearing B cells were comparable in well nourished and malnourished subjects but the proportion of B was increased in the latter Null cells without the conventional markers of T or B cells were proportionately increased Leukocyte terminal deoxynucleotidyl transferase activity was elevated in the majority of undernourished children and correlated with the proportion of null cells The significance of these observations is discussed and it is suggested that null cells represent immature undifferentiated T lymphocytes

KEY WORDS Malnutrition T lymphocytes B lymphocytes terminal deoxynucleotidyl transferase null cells cellular immunity

Nutritional deficiency enhances susceptibility to infectious disease (9 24) Besides the common causal factors of poverty poor sanitation and personal hygiene overcrowding and contaminated food and water undernutrition impairs several facets of the immune response (10 11) thereby increasing vulnerability to disease produced by pathogenic microorganisms Depression of cell mediated immunity is a common observation as evidenced by reduced cutaneous delayed hypersensitivity to ubiquitous recall antigens and sensitizing chemicals decreased mitogen induced lymphocyte proliferation response and low numbers of rosette forming T lymphocytes in the peripheral blood (3–6 12 14 20 21 23) Recent immunologic work has demonstrated the heterogeneity of human T lymphocytes with regard to cell surface receptors histochemical characteristics RNA content response to phytomitogens sensitivity to corticosteroids and irradiation distribution in organs chemotaxis lymphokine production and immunoregulatory role (25) Alterations in the proportion of lymphocyte subsets have been reported in primary immunodeficiency (28) and in malignancy (25) There is no published report on changes in lymphocyte subpopulations of T and B cells in malnutrition in man or experimental animals

MATERIAL AND METHODS

Subjects

Twenty-one young children aged 11–38 months were diagnosed to be suffering from energy protein undernutrition on the basis of clinical features (growth failure loss of subcutaneous tissue skin and hair changes) decreased weight for height and low serum transferrin less than 16 mg% (7 11) All the patients came from rural India and none had any obvious systemic disease

Fifteen healthy subjects aged 12–36 months from the same community served as controls

Mononuclear cells

Heparinized peripheral venous blood was collected and mononuclear cells were isolated by Ficoll Hypaque den

Table 1 *Lymphocyte subpopulations and levels of terminal deoxynucleotidyl transferase (TdT)*
Values are given as mean ± S E M Significance of differences (p) was assessed statistically using Student s t test NS = not significant

Group	No	Total leukocyte count (per mm³)	Rosette forming T cells (%)	B cells (%) B	B cells (%) B_γ	B cells (%) B_μ	Null cells (%)	TdT (units/10⁸ cells)
Undernourished	21	6 852±1 471	47 3±3 9	9 1±1 1	4 2±0 8	5 7±0 9	30 7±2 6	11 34±2 42
Healthy	15	7 950±1 894	73 7±2 8	4 0±0 7	6 3±0 7	8 7±1 2	7 0±0 7	1 08±0 0[illegible]
p		NS	<0 01	<0 05	NS	NS	<0 001	<0 001

sity gradient centrifugation Cells were washed twice in Hank s balanced salt solution and resuspended in RPMI 1640 containing 15% heat inactivated fetal calf serum Phagocytic cells were depleted by addition of lymphocyte separation reagent (Technicon) incubation at 37°C rotator for 30 min and centrifugation at 400 g for 20 min Interface cells were washed twice with Hank s balanced salt solution and resuspended in RPMI 1640 at a concentration of 4 × 10⁶ cells per ml

T lymphocytes
Lymphoid cells were mixed with equal volume of 1% neuraminidase treated sheep red blood cells (SRBC) in 25% fetal calf serum The mixture was incubated at 37°C for 5 min centrifuged at 200 g for 5 min and incubated again at 4 C for 1 hour The cell pellets were resuspended by gentle agitation kept at 4 C for 10 min and examined for the proportion of cells forming rosettes with SRBC

Aliquots of rosetting T lymphocytes were separated from non T cells by density gradient centrifugation SRBC were lysed with distilled water and MEM T lymphocyte subpopulations were separated by employing ox red blood cells (ORBC) coated with anti ORBC antibodies of IgM or IgG class Isolated T lymphocytes were mixed with an equal volume of 1% ORBC anti ORBC IgM for enumeration of T_μ cells or ORBC anti ORBC IgG for enumeration of T_γ cells centrifuged at 200 g for 5 min and incubated at 4 C for 1 hour The cell pellets were resuspended by gentle agitation kept at 4 C for 10 min and examined for the proportion of cells forming rosettes with ORBC

B lymphocytes
Cells bearing surface membrane immunoglobulin (smIg) were recognized by speckled or crescent shaped fluorescence on staining with fluorescein conjugated goat antiserum raised against human IgG IgA and IgM prepared in our laboratory (6 28) Mononuclear cells were incubated in culture medium at 37°C for one hour and then incubated with the conjugated antisera After thorough washing the cell preparations were examined under a fluorescence microscope equipped with epi illumination Monocytes were identified and excluded by their ability to phagocytose latex particles Furthermore duplicate sets of cell preparations were treated with trypsin incubated in culture medium for 8 hours and restained to demonstrate newly produced immunoglobulin

Terminal deoxynucleotidyl transferase (TdT)
Mononuclear cells were ruptured by freezing and thawing three times The suspension was centrifuged at 80000 g for 1 hour and the supernatant assayed for TdT activity by the method of Coleman et al (13) The results were expressed in units of 10 cells one unit of activity being the polymerisation by TdT of 1 nmol of nucleotide in 1 hour

RESULTS

The number of T lymphocytes was lower and the proportion of smIgA bearing B cells and null cells was increased in undernourished children compared with findings in well nourished matched controls (Table 1) These differences were statistically significant The total number of leukocytes and the proportion of B cells were comparable in the two groups The Bα cells were increased in the malnourished

Th lymphocytes with receptors for IgM were decreased (p<0 1) and those with receptors for IgG were increased (0 05<p<0 1) in malnourished subjects (Fig 1)

Leukocyte TdT activity was significantly higher in undernourished children (p<0 001) and correlated with the proportion of null cells in the peripheral blood (r=0 78 p<0 01) (Fig 2 Table 1)

DISCUSSION

We observed a marked increase in leukocyte TdT (terminal deoxynucleotidyl transferase DNA nucleotidylexo transferase nucleoside triphosphate DNA deoxynucleotidyl trans

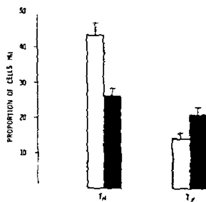


Fig 1 T lymphocyte subpopulations with cell surface receptors for IgM (T_μ) and IgG (T_γ). Values are expressed as proportion of total T cell number and given as mean ±S D Blocked columns represent undernourished children and open columns matched healthy controls

ferase E C 2 7 7 31) in energy protein undernutrition This enzyme is restricted in distribution to the thymus bonemarrow and peripheral blood of a number of animal species (2 27) which has been interpreted to indicate that it is involved in the functional differentiation of T cells and generation of immunological diversity and memory (2) It has been postulated that TdT may act as a somatic mutagen in lymphocytes (1) The intracellular location of the enzyme varies with the phase of the cell cycle with a prominent shift from the nucleus to cytoplasm during mitosis In the thymus TdT positive cells are restricted to the cortex There is a heterogeneity in the amount of the enzyme within different cells as demonstrated by immunofluorescence or biochemical assay (15) The majority of high and low density thymic cells contain little or no TdT whereas almost all medium density cortical thymocytes are TdT positive It is possible that some cells contain antigenically intact but functionally inactive enzyme The critical role of the thymus in the differentiation of TdT positive lymphocytes is demonstrated by low enzyme activity in congenitally athymic nude (nu/nu) mice and in neonatally thymectomized mice Thymosin has been shown to induce TdT activity in bone marrow cells of nude mice (22) The contribution of thymus derived cells to the pool of TdT positive bone marrow cells is perhaps negligible (19) It is fascinating to note that both TdT positive and thymic antigen positive cells arise almost simultaneously during ontogenetic development although there are differences in the rate of evolution of these markers

This study has demonstrated that nutritional deficiency is associated with a marked alteration in the number of lymphocyte subpopulations The total number of smIg bearing B lymphocytes was comparable in well nourished and malnourished groups although it was somewhat higher than the figures obtained in healthy well nourished subjects examined in Europe and North America These differences may reflect the more frequent exposure of our study subjects to various microorganisms as a result of poor sanitation and heavily contaminated environment including water and food The proportion of B cells bearing α heavy chain of immunoglobulin (B lymphocytes) was increased in the malnourished group

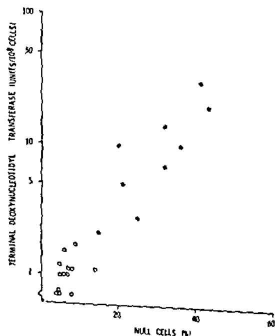


Fig 2 Leukocyte TdT activity related to the number of null cells in undernourished (●) and healthy (○) groups

Table I *Lymphocyte subpopulations and levels of terminal deoxynucleotidyl transferase (TdT)*

Values are given as mean ± S E M Significance of differences (p) was assessed statistically using Student's t test NS = not significant

Group	No	Total leukocyte count (per mm³)	Rosette forming T cells (%)	B cells (%)			Null cells (%)	TdT (units/10⁸ cells)
				B_α	B_γ	B_μ		
Undernourished	21	6 852±1 471	47 3±3 9	9 1±1 1	4 2±0 8	5 7±0 9	30 7±2 6	11 34±2 42
Healthy	15	7 950±1 894	73 7±2 8	4 0±0 7	6 3±0 7	8 7±1 2	7 0±0 7	1 08±0 0
p		NS	<0 01	<0 05	NS	NS	<0 001	<0 001

sity gradient centrifugation Cells were washed twice in Hank's balanced salt solution and resuspended in RPMI 1640 containing 15% heat inactivated fetal calf serum Phagocytic cells were depleted by addition of lymphocyte separation reagent (Technicon) incubation at 37°C rotator for 30 min and centrifugation at 400 g for 20 min Interface cells were washed twice with Hank's balanced salt solution and resuspended in RPMI 1640 at a concentration of 4 × 10⁶ cells per ml

T lymphocytes

Lymphoid cells were mixed with equal volume of 1% neuraminidase treated sheep red blood cells (SRBC) in 25% fetal calf serum The mixture was incubated at 37°C for 5 min centrifuged at 200 g for 5 min and incubated again at 4 C for 1 hour The cell pellets were resuspended by gentle agitation kept at 4 C for 10 min and examined for the proportion of cells forming rosettes with SRBC

Aliquots of rosetting T lymphocytes were separated from non T cells by density gradient centrifugation SRBC were lysed with distilled water and MEM T lymphocyte subpopulations were separated by employing ox red blood cells (ORBC) coated with anti ORBC antibodies of IgM or IgG class Isolated T lymphocytes were mixed with an equal volume of 1% ORBC anti ORBC IgM for enumeration of T_μ cells or ORBC anti ORBC IgG for enumeration of T_γ cells centrifuged at 200 g for 5 min and incubated at 4 C for 1 hour The cell pellets were resuspended by gentle agitation kept at 4 C for 10 min and examined for the proportion of cells forming rosettes with ORBC

B lymphocytes

Cells bearing surface membrane immunoglobulin (smIg) were recognized by speckled or crescent shaped fluorescence on staining with fluorescein conjugated goat anti serum raised against human IgG IgA and IgM prepared in our laboratory (6 28) Mononuclear cells were incubated in culture medium at 37°C for one hour and then incubated with the conjugated antisera After thorough washing the cell preparations were examined under a fluorescence microscope equipped with epi illumination Monocytes were identified and excluded by their ability to phagocytose latex particles Furthermore duplicate sets of cell preparations were treated with trypsin incubated in culture medium for 8 hours and restained to demonstrate newly produced immunoglobulin

Terminal deoxynucleotidyl transferase (TdT)

Mononuclear cells were ruptured by freezing and thawing three times The suspension was centrifuged at 80 000 g for 1 hour and the supernatant assayed for TdT activity by the method of Coleman et al (13) The results were expressed in units of 10 cells one unit of activity being the polymerisation by TdT of 1 nmol of nucleotide in 1 hour

RESULTS

The number of T lymphocytes was lower and the proportion of smIgA bearing B cells and null cells was increased in undernourished children compared with findings in well nourished matched controls (Table I) These differences were statistically significant The total number of leukocytes and the proportion of B cells were comparable in the two groups The $B\alpha$ cells were increased in the malnourished

The lymphocytes with receptors for IgM were decreased (p<0 1) and those with receptors for IgG were increased (0 05<p<0 1) in malnourished subjects (Fig 1)

Leukocyte TdT activity was significantly higher in undernourished children (p<0 001) and correlated with the proportion of null cells in the peripheral blood (r=0 78 p<0 01) (Fig 2 Table I)

DISCUSSION

We observed a marked increase in leukocyte TdT (terminal deoxynucleotidyl transferase DNA nucleotidylexo transferase nucleoside triphosphate DNA deoxynucleotidyl trans

13 Coleman M S Greenwood M F Hutton J J Bollum F J Lampkin B & Holland P Serial observations on terminal deoxynucleotidyl transferase activity and lymphoblast surface markers in acute lymphoblastic leukemia *Cancer Res 36* 1?0 1976
14 Ferguson A S Lawlor G J Jr Neumann C G Oh W & Stiehm E R Decreased rosette forming lymphocytes in malnutrition and intrauterine growth retardation *J Pediatr 85* 717 1974
15 Goldschneider I Gregorie K E Barton R W & Bollum F J Demonstration of terminal deoxy nucleotidyl transferase in thymocytes by immuno-fluorescence *Pr c Natl Acad Sci USA 74* 734 1977
16 Gross R L Reid J V O Newberne P M Burgess B Marston R & Hift W Depressed cell mediated immunity in megaloblastic anemia due to folic acid deficiency *Am J Clin Nutr 28* ??5 1975
17 Gupta S Fernandes G Nair M & Good R A Spontaneous and antibody dependent cell mediated cytotoxicity by human T cell subpopulations *Proc Natl Acad Sci USA 75* 5137 1978
18 Harland P S E G Tuberculin reactions in mal nourished children *Lancet I* 719 1965
19 Hutton J J & Bollum F J Terminal deoxy nucleotidyl transferase (TdT) in marrow of athymic nude mice *Blood 48* 1003 1976
0 MacDougall L G Anderson R McNab G M & Katz J Immune response in iron-deficient children impaired cellular defense mechanisms with altered humoral components *J Pediatr 86* 833 1975
1 Neumann C G Lawlor C G Steihm E R Swedseid M E Newton C Herbert J Ammann A J & Jacob M Immunological responses in mal nourished children *Am J Clin Nutr 28* 89 1975
2? Pazmino N H Ihle J N & Goldstein A L Introduction *in vivo* and *in vitro* of terminal deoxy nucleotidyl transferase by thymosin in bone marrow cells from athymic mice *J Exp Med 147* 708 1978
?3 Reddy V Bhaskaram C & Raghuramulu N Immunological responses in malnourished children *Indian Pediatr 14* ?55 1977
?4 Scrimshaw N S Taylor C E & Gordon J E *Interactions of nutrition and infection* World Health Organization Geneva 1968
25 Serrou B & Rosenfeld C *Human lymphocyte differentiation Its application to human cancer* North Holland Amsterdam 1978
6 Smythe P M Breton Stiles G G Grace H J Mafoyane A Schonland M Coovadia H M Loening W E K Parent M A & Vos G H Thymolymphatic atrophy and depression of cell mediated immunity in protein-calorie malnutrition *Lancet II* 939 1971
?7 Sugimoto M & Bollum F J Terminal deoxy nucleotidyl transferase (TdT) in chick embryo lymphoid tissues *J Immunol 122* 39 1979
28 WHO Scientific Group *Immunodeficiency* Tech Rep Ser 630 World Health Organization Geneva 1978

Submitted March 6 1979
Accepted April 23 1979

Department of Nutrition and Food Science
Massachusetts Institute of Technology
Cambridge Mass 0 139
USA

which may be the result of repeated gastro intestinal and respiratory infections in undernourished children

There was a significant decrease in the number of T lymphocytes with cell surface receptors for IgM (T_μ) in malnutrition (Fig 1) These cells exert helper function in the differentiation of B lymphocytes to plasma cells On the other hand the proportion of T cells with receptors for IgG (T_γ) was increased This subset of lymphocytes has recently been shown to mediate spontaneous lymphocyte mediated cytotoxicity and antibody dependent cell mediated cytotoxicity (17) Null cells also exert spontaneous lymphocyte mediated cytotoxicity Both these activities had been demonstrated by us to be increased in malnourished children (6) in observation compatible with our present data of increase in T_γ and null cell populations (Fig 1 Table 1) The biological significance of these changes in T cell subsets are not yet clear Nevertheless such alterations have been noted in primary immunodeficiency (26) and in cancer (25)

There was a significant reduction in the number of rosette forming T lymphocytes This may be the result of a number of factors Reduced cellular regeneration is the hallmark of protein deficiency (7) and may well be reflected in impaired proliferation of lymphoid cells The frequent increase in free cortisol not bound to albumin in malnutrition may actually produce a lympholytic effect T cells are susceptible to the influence of corticosteroids

An alternative explanation for the reduction of rosetting T lymphocytes may lie in impaired maturation of T cell precursors The various sequential steps of differentiation from progenitor T cell to mature T lymphocyte have been delineated recently (28) The acquisition of cell surface markers including the ability to rosette with SRBC is a relatively late event in differentiation The marked increase in the proportion of null cells and a corresponding increase in leukocyte TdT activity suggests that many of the null cells are immature T lymphocytes TdT is found almost exclusively in immature T lymphocytes and is progressively lost as the cell differentiates to full maturity TdT is also increased in acute lymphoblastic leukemia in which the blast cell has many of the morphological histochemical and surface membrane characteristics of immature lymphocytes This is particularly true of null blast leukemia (R K Chandra unpublished data) The hypothesis that the reduction in rosetting T lymphocytes increase in null cells and elevated TdT activity in nutritional deficiency all point to impaired maturation of T lymphocytes is further supported by decreased thymic hormone levels in the serum of malnourished children (10a)

REFERENCES

1 Baltimore D Is terminal deoxynucleotidyl transferase a somatic mutagen in lymphocytes? *Nature* 248 409 1974

2 Bollum F J Terminal deoxynucleotidyl transferase *In* P D Boyer (ed) *The enzymes* Academic Press New York 1974 vol 10 p 145

3 Chandra R K Immunocompetence in undernutrition *J Pediatr 81* 1184 1972

4 Chandra R K Rosette forming T lymphocytes and cell mediated immunity in malnutrition *Br Med J III* 608 1974

5 Chandra R K Fetal malnutrition and postnatal immunocompetence *Am J Dis Child 129* 450 1975

6 Chandra R K Lymphocyte subpopulations in malnutrition Cytotoxic and suppressor cells *Pediatrics 59* 423 1977

Chandra R K Energy protein undernutrition *In* V C Kelley (ed) *Practice of pediatrics* Harper & Row Hagerstown 1977 ch 32 p 1

8 Chandra R K & Matsumura T Ontogenetic development of the immune system and effects of fetal growth retardation *J Perinat Med 7* 1979 In press

9 Chandra R K Nutritional deficiency and susceptibility to infection *Bull WHO 57* 167 1979

10 Chandra R K Interactions of nutrition infection and immune response Immunocompetence in nutritional deficiency methodological considerations and intervention strategies *Acta Paediatr Scand 68* 137 1979

10a Chandra R K Serum thymic hormone activity in protein energy malnutrition *Clin Exp Immunol* 1979 in press

11 Chandra R K & Newberne P M *Nutrition immunity and infection Mechanisms of interactions* Plenum New York 1977

12 Chandra R K Au B Woodford G & Hyam P Iron status immunocompetence and susceptibility to infection In *Ciba Foundation Symposium on Iron Metabolism* Elsevier Amsterdam 1977 p 249

13 Coleman M S Greenwood M F Hutton J J Bollum F J Lampkin B & Holland P Serial observations on terminal deoxynucleotidyl transferase activity and lymphoblast surface markers in acute lymphoblastic leukemia *Cancer Res 36* 1 0 1976
14 Ferguson A S Lawlor G J Jr Neumann C G Oh W & Stiehm E R Decreased rosette forming lymphocytes in malnutrition and intrauterine growth retardation *J Pediatr 85* 717 1974
15 Goldschneider I Gregorie K E Barton R W & Bollum F J Demonstration of terminal deoxy nucleotidyl transferase in thymocytes by immunofluorescence *Proc Natl Acad Sci USA 74* 734 1977
16 Gross R L Reid J V O Newberne P M Burgess B Marston R & Hift W Depressed cell mediated immunity in megaloblastic anemia due to folic acid deficiency *Am J Clin Nutr 28* 225 1975
17 Gupta S Fernandes G Nair M & Good R A Spontaneous and antibody dependent cell mediated cytotoxicity by human T cell subpopulations *Proc Natl Acad Sci USA 75* 5137 1978
18 Harland P S E G Tuberculin reactions in malnourished children *Lancet I* 719 1965
19 Hutton J J & Bollum F J Terminal deoxy nucleotidyl transferase (TdT) in marrow of athymic nude mice *Blood 48* 1003 1976
20 MacDougall L G Anderson R McNab G M & Katz J Immune response in iron-deficient children impaired cellular defense mechanisms with altered humoral components *J Pediatr 86* 833 1975
21 Neumann C G Lawlor C G Steihm E R Swedseid M E Newton C Herbert J Ammann A J & Jacob M Immunological respon es in malnourished children *Am J Clin Nutr* 8 89 1975
22 Pazmino N H Ihle J N & Goldstein A L Introduction *in vivo* and *in vitro* of terminal deoxy nucleotidyl transferase by thymosin in bone marrow cells from athymic mice *J Exp Med 147* 708 1978
23 Reddy V Bhaskaram C & Raghuramulu N Immunological responses in malnourished children *Indian Pediatr 14* 255 1977
24 Scrimshaw N S Taylor C E & Gordon J E *Interactions of nutrition and infection* World Health Organization Geneva 1968
25 Serrou B & Rosenfeld C *Human lymphocyte differentiation Its application to human cancer* North Holland Amsterdam 1978
26 Smythe P M Breton Stiles G G Grace H J Mafoyane A Schonland M Coovadia H M Loening W E K Parent M A & Vos G H Thymolymphatic atrophy and depression of cell mediated immunity in protein-calorie malnutrition *Lancet II* 939 1971
27 Sugimoto M & Bollum F J Terminal deoxy nucleotidyl transferase (TdT) in chick embryo lymphoid tissues *J Immunol 122* 392 1979
28 WHO Scientific Group *Immunodeficiency* Tech Rep Ser 630 World Health Organization Geneva 1978

Submitted March 6 1979
Accepted April 23 1979

Department of Nutrition and Food Science
Massachusetts Institute of Technology
Cambridge Mass 02139
USA

Acta Pædiatr Scand 68 847–852 1979

THE SPECTRUM OF COW'S MILK ALLERGY IN CHILDHOOD

Clinical Gastroenterological and Immunological Studies

D J HILL G P DAVIDSON D J S CAMERON and G L BARNES

From the Department of Gastroenterology Royal Children s Hospital Melbourne Australia

ABSTRACT Hill D J Davidson G P Cameron D J S and Barnes G L (Department of Gastroenterology Royal Children s Hospital Melbourne Australia) The spectrum of cow s milk allergy in childhood Clinical gastroenterological and immunological studies Acta Paediatr Scand 68 847 1979 —Seventeen of 52 children suspected of having cow s milk allergy had this diagnosis confirmed after milk challenge in hospital A broad spectrum of reactions was observed including skin eruptions respiratory symptoms and gastrointestinal disturbance Not all patients with gastrointestinal symptoms showed small bowel mucosal damage Only patients with skin reactions had positive skin tests IgA deficiency and IgE elevation were common Four patients had symptoms within 3 days of birth Twelve children tolerated cow s milk by three years of age Cow s milk allergy can cause a variety of symptoms Challenge with milk for several days may be required before allergic manifestations can be demonstrated

KEY WORDS Cow s milk allergy gastrointestinal tract skin

Allergy to cow s milk has been attributed to an immunological hypersensitivity to several proteins (22) There is evidence that specific humoral (19 21) and cellular (3 4) immune mechanisms may operate in cow s milk allergy (CMA) although their importance in the pathogenesis of milk hypersensitivity in individual patients is poorly understood Several well defined syndromes have been associated with CMA (7 10 16 31) but the multiplicity of poorly defined illnesses (7) the reported variability of the pathological lesion following milk ingestion in sensitized children (6 16 24) and the lack of a diagnostic laboratory test have led to either a reluctance to recognize CMA (9) or confusion with other more common causes of mild intolerance (5)

This study was designed to establish the spectrum of clinical disorders affecting the skin respiratory and gastrointestinal tracts and to measure some immunological phenomena associated with CMA As in other studies the diagnosis of CMA has rested on a reproducible response to cow s milk challenge symptomatic improvement on withdrawal of whole cow s milk protein from the diet and exclusion of other known causes of milk intolerance

PATIENTS AND METHODS

The children whose investigations are reported here come from a group of 52 children who had gastrointestinal cutaneous or respiratory symptoms attributed to CMA by their parents and/or referring physician

Initial outpatient investigations identified an alternative diagnosis in eight cases (suger intolerance infection infestation coeliac disease or milk aspiration) The remaining 44 children were admitted to hospital for investigation Twenty-seven of them showed no response to milk challenge and were excluded from further consideration The remaining 17 children reacted to cow s milk challenge in hospital and form the basis of this report Eleven patients were breast fed for 3 to 14 months Five were given only artificial feeds (patients 4 6 8 1 and 16) and one (patient 5) had mixed feeds

Each child was stabilised on a diet free from whole cow s milk proteins for at least four weeks before admission The protocol followed is outlined in Fig 1 and has been previously described (5) The symptomatic response to milk provocation was d fined as follows immediate reactions occurred within one hour intermediate reactions between one and 24 hours and late reactions greater than 24 hours after commencing milk challenge Patients with late reactions were ingesting normal volumes of cow s milk for their age at the time symptoms developed

only a post challenge biopsy The method used was that of Townley and Barnes (6) Duodenal juice and mucosal impression smears were examined for trophozoites of *Giardia lamblia* and tissue was prepared for histological examination and disaccharidase estimation (?7) Structural changes were graded as showing normal appearance mild moderate or severe change as described by Townley et al (7)

Other investigations Patients with respiratory symptoms associated with cow s milk ingestion had a barium swallow to detect aspiration (30)

RESULTS

Symptomatic response to formal milk provocation Eight of the 17 patients developed symptoms primarily involving the gastro intestinal tract but in only 3 was the reaction immediate (Table 1) Seven patients developed mainly skin reactions and this response was immediate in all Three of the seven also developed diarrhoea and/or vomiting Most cutaneous eruptions resolved within hours but in one child (case 10) the rash persisted for 72 hours Respiratory symptoms were pre dominant in two of the 17 children cough chest rattle and wheeze followed milk challenge Two other patients (cases 9 and 10) gave a history of acute respiratory distress and skin eruptions following ingestion of milk at home but failed to reproduce these symptoms when challenged Urticaria was elicited in these 2 patients after a small volume of milk was given and the challenge procedure ceased

Four of the 17 children had a past history of pallor and shock consistent with an ana phylactic reaction Two (patients 4 and 7) developed mainly gastrointestinal symptoms at the time of challenge the other two had an immediate skin reaction as well as vomiting and/or diarrhoea

Immune function studies The results of

a tase ≥1 1 units/g wet weight)		Skin test to milk	RAST to milk	IgE (IU/ml)	Other immune abnormalities	Other food allergies	Age of milk tolerance (months)
efore	After						
3	4 7	Neg	Neg	-		Nil	?
5	5 0	Neg	Neg	943	IgA<5th ~	Nil	4
	4 5	Neg	Neg	40		Nil	0
3	1 3	Neg	Neg	0		Nil	16
	0	Neg	Neg	-		Nil	16
1	1 1	Neg	Neg	-		Soy	36
1	0 3	Neg	Neg	15		Nil	Intolerant
6	1 6	Neg	Neg	<10	IgA<5th ~	Soy	Intolerant
4	6	Pos	Pos	-		Egg	Intolerant
9		Pos	Pos	158	IgA<5th ~	Egg	13
1 7	5 8	Pos	Neg	46	IgA none detected	Nil	10
1 9	9	Pos	Neg	14	IgA<5th ~	Nil	15
	-	Pos	Neg	381	IgA none detected	Egg Lamb	Intolerant
1 5	0	Pos	Neg	-	IgG>95th ~	Egg	18
1	4	Pos	Pos	-	IgG<5th ~	Egg Lamb Peanut	Intolerant
1 0	0 5	Neg	Neg	100		Nil	36
	-			-		Nil	30

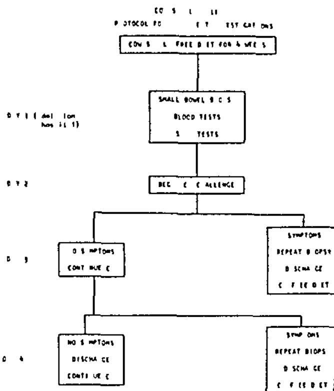


Fig 1 Protocol for inpatient investigation of patients with suspected CMA

Milk challenge Commercially pasteurized cow s mill was used in an initial dose of 0 5 to 5 ml If no respons occurred within 30 min the volume was doubled at hourl intervals until a diet containing normal volumes of cow s milk for weight was established or a positive response was elicited

Skin tests Prick testing with whole cow s milk extract (Bencard U K) and appropriate controls was performed (5) The results were read at 15 min and the wheal mea sured A diameter of less than 2 mm was read as nega tive

Immune function studies Prior to challenge blood was taken by venepuncture for full blood examination immunoglobulins G A M and E and IgE cow s milk allergen specific antibodies Immunoglobulins G A and M were estimated by a modification of the Mancini method (17) and the results expressed as a percentile for age (23) Total IgE was measured by the method of Wide & Porath (29) Allergen specific IgE antibodies to cow s milk were measured using the Radioallergosorbent Test (RAST) (13) and results expressed as positive or nega tive A negative score was recorded when the count was less than that of the control

Duodenal biopsy Thirteen of the 17 patients had a biopsy before and after milk challenge Post challenge biopsy was taken within 24 hours of the onset of symp toms One had only a prechallenge biopsy and one had

Table 1 *Clinical features and responses to milk challenge in 17 patients with cow s milk aller grouped according to predominant reaction*

– indicates test not carried out Histological abnormality N=normal Mild Moderate and Severe as described by Town et al (19) Presenting symptoms V=vomiting d=diarrhoea AE=angio oedema A=anaphylaxia FTT=failure to thr U=urticaria

Patient	Age of first symptoms (months)	Age of diagnosis (months)	Presenting symptoms	Symptoms following milk challenge	Onset of reaction	Histological abnormality Before	After
Gastrointestinal							
1	4	8	V D AE	D	Late	N	N
2	14	17	V D Pallor	D V	Late	N	N
3	3	15	D	D	Late	N	N
4	Day 3	5	V D A	V D Pallor	Immediate	Mild	Moderat
5	2	10	D FTT	V D	Late	–	Mild
6	Day 12	10	V Bloody D FTT	V D	Immediate	N	N
7	3	7	V D A	V D Pallor	Immediate	N	Severe
8	Day 1	16	V D	V D	Intermediate	N	Moderat
Skin							
9	4	12	U AE Cough Tachypnoea D	U AE	Immediate	Mild	Mild
10	3	4	U Rash Pallor AE Cough	U	Immediate	N	–
11	Day 1	4	Rash AE D	Rash AE	Immediate	N	Mild
12	7	7	U AE V D A	U AE V D	Immediate	N	N
13	Day 18	6	U V Pallor Irritability	U Pallor Irritability	Immediate	–	–
14	1½	11	U D FTT	Rash D	Immediate	Mild	Mild
15	1	11	U V A	V A Rash	Immediate	N	N
Respiratory							
16	Day 2	24	Wheeze D	Wheeze	Late	Mild	Mild
17	7	17	Wheeze Cough	Wheeze Cough	Intermediate	–	–

Skin sensitizing antibodies were found only in patients with cutaneous eruptions but not all of these had circulating IgE milk antibodies detected. Possibly short latency IgG antibodies (21) or incomplete saturation of peripheral IgE receptors accounts for these findings. Skin sensitizing antibodies often associated with anaphylactic reactions to allergens were detected in two of the children who had an anaphylactic reaction to cow's milk. However two others had no cutaneous reaction at the time of anaphylaxis nor at the time of formal milk challenge. Neither skin nor circulating IgE milk antibodies were present in the latter 2 cases. There is evidence that IgE mucosal hypersensitivity to allergens can occur without systemic or cutaneous involvement (11) but the possibility that other immune mechanisms may be operating in anaphylaxis cannot be excluded.

In a previous report (5) we attributed respiratory symptoms after milk ingestion to aspiration (30). Four patients in this study had a history of repeated respiratory symptoms after ingestion of cow's milk but not after soy formulae or other fluids. In 2 of the 4 wheezing occurred for several hours after challenge in hospital and this was reproduced on 3 occasions.

A marked discrepancy was noted between the severe symptoms reported by parents and those elicited by formal milk challenge. In hospital only the smallest volume of milk which elicited a response was given and the challenge ceased. In all cases this challenge volume of milk was much less than that given by parents at home. A variation in the volume of milk given in milk challenge by different investigators may account for the conflicting reports regarding the nature, rate of onset, frequency and severity of symptoms in CMA as well as differences in mucosal damage (6, 16, 24).

It has been suggested that breast feeding may prevent the development of some allergic diseases (18). Eleven of our patients were breast fed and had reactions after the first known milk exposure. Two children (patients 8 and 11) had severe illnesses consistent with CMA within hours of birth and CMA was confirmed by challenge at 3 months of age. Possibly hypersensitivity to cow's milk may develop in utero (14) or cow's milk protein may be transmitted in breast milk (12). By the third year of life most children were able to ingest volumes of milk which previously caused symptoms. Some showed a dislike for milk but did not appear to continue to suffer from CMA. However CMA may persist beyond this age (8).

This study has clearly shown that there is no single laboratory test on which to base the diagnosis of CMA and that the diagnosis still rests on a reproducible response to challenge under controlled conditions. Small bowel biopsy has an important role in excluding other non immune causes of gastrointestinal milk intolerance. It also provides a baseline for interpreting post challenge mucosal changes in patients with gastrointestinal symptoms in whom the response to milk is uncertain. Where a clear cut cutaneous, respiratory or gastrointestinal response occurs after milk challenge a repeat small bowel biopsy appears to add little to the management of this disorder.

ACKNOWLEDGEMENTS

D. J. H. was supported by the National Health and Medical Research Council.

We thank Dr C. S. Hosking, Immunologist, Department of Pathology, Royal Children's Hospital, Parkville, for the immunological studies, Mrs Rosa Kay and Mr Max Murray for technical assistance and Mrs Anne Peace for secretarial assistance.

REFERENCES

1. Ammann A. J. & Hong R. Selective IgA deficiency. Presentation of 30 cases and a review of the literature. *Med cine* 50 2–3 1971
2. Barnes G. L. & Townley R. R. W. Duodenal mucosal damage in 31 infants with gastroenteritis. *Arch Dis Child* 48 343 1973
3. Borrone C., Dagna Bricarelli F., Massimo L., Fossati-Guglielmoni A. & Durand P. Lymphocyte transformation in milk allergy. *Acta Paediat Scand* 59 449 1970

skin and RAST tests to cow s milk are shown in Table 1 No patient with predominantly gastrointestinal symptoms had evidence of skin sensitivity or circulating IgE antibodies to cow s milk All seven patients who developed a skin eruption after milk ingestion had skin sensitizing antibodies present as evidenced by positive skin tests and 4 also had circulating IgE milk antibodies One patient (case 16) with respiratory symptoms had skin sensitivity but not circulating IgE milk antibodies demonstrated The other patient (case 17) had neither skin sensitivity nor circulating milk antibodies

Six children had serum IgA levels below the 5th percentile for age and 1 patient had a serum IgG level below the 5th percentile (23) Total IgE was at least 100 IU/ml in 5 of the 10 patients in whom it was measured

Haemoglobin levels and eosinophil counts were within the normal range in all patients

Studies of gastrointestinal structure and function Of the 8 children with predominantly gastrointestinal symptoms 4 showed mucosal damage after milk challenge (Table 1) The mucosal abnormality was mild in 1 (case 5) moderate in 2 (cases 4 and 8) and severe in 1 (case 7) One of these patients (case 4) had mild mucosal abnormalities prior to challenge which became more marked after challenge Only 1 of the 6 children with mainly cutaneous manifestations of CMA (case 11) showed duodenal mucosal changes after milk challenge Two others (cases 9 and 11) had mild mucosal changes present before and after challenge

One child (case 16) with predominantly respiratory symptoms had associated gastrointestinal symptoms His duodenal mucosa showed mild non specific abnormalities before and after milk challenge

Lactase depression to half the prechallenge level occurred in 4 children with gastrointestinal symptoms (patients 4 6 7 and 8) after milk challenge The only child with respiratory symptoms to have a biopsy (case 16) had lactase depression noted before and after challenge His gastrointestinal and re-spiratory symptoms settled when fed wit[h] Glucose Nutramigen (Mead Johnson)

Age of onset and duration of CMA Fou[r] children had symptoms consistent with CM[A] in the first 72 hours of life (cases 4 8 11 an[d] 16) at the time of first milk ingestion in th[e] neonatal nursery Six other children ha[d] symptoms of CMA at the time of first know[n] milk ingestion (cases 2 7 10 13 14 and 15) Their ages at this time ranged from 18 day[s] to 14 months

Twelve of the 17 patients became tolera[nt] to cow s milk between 10 and 36 months [of] age (Table 1) Five patients were still unabl[e] to tolerate cow s milk at 7 to 28 months [of] age

Associated food allergy Soy egg or lam[b] hypersensitivity was seen in association wit[h] CMA in 7 patients (Table 1)

DISCUSSION

Gastrointestinal respiratory and cutaneo[us] reactions have each been described in cow milk protein hypersensitivity (7 10 31) A[n] association between IgA deficiency and hi[gh] IgE levels (1 15) and the limitations of sk[in] testing to diagnose CMA (5) have been r[e]ported This paper describes the spectrum [of] these associations in 17 children with prove[n] CMA

In contrast to other reports (25) small bow[el] mucosal damage was not seen in all patien[ts] with gastrointestinal symptoms This may b[e] because the lesion in CMA is patchy as i[n] gastroenteritis (2) or detectable only by ele[c]tronmicroscopy (24) but the degree of muc[o]sal damage is also likely to be related to th[e] amount of antigen administered during cha[l]lenge

Little attention has been given to th[e] gastrointestinal symptoms which develop 2[4] hours or more after commencing cow s mil[k] ingestion (16) Such late reactions occurred i[n] 4 of our patients emphasizing the need t[o] continue cow s milk challenge for several day[s] in children with suspected CMA

Acta Pædiatr Scand 68 853–859 1979

A PROSPECTIVE STUDY OF COW S MILK PROTEIN INTOLERANCE IN SWEDISH INFANTS

I JAKOBSSON and T LINDBERG

From the Department of Paediatrics Malmo General Hospital Malmo Sweden

ABSTRACT Jakobsson I and Lindberg T (Department of Paediatrics Malmo General Hospital Malmo Sweden) A prospective study of cow s milk protein intolerance in Swedish infants Acta Paediatr Scand 68 853 1979 —1079 of 1548 newborn infants were followed during their first year 328 were prospectively contacted once a month 751 were followed up at child welfare clinics Altogether 20 were diagnosed as being cow s milk protein intolerant (1 9%) Symptoms from the gastrointestinal tract and the skin predominated Only 2 had respiratory symptoms Ten had their symtpoms within one week after the introduction of cow s milk 3 of them at their first cow s milk-containing meal A further 4 already had symptoms when fed only human milk The others (6 infants) showed symptoms after more than one week on a cow s milk containing diet Before 2 years of age 13 had recovered Twelve of the cow s milk protein intolerant infants also showed adverse reactions to other foods soy protein intolerance being the most common (7 infants) A family history of allergy was found in 35% (116) of the 328 infants and in 70% (14) of those with cow s milk protein intolerance

KEY WORDS Cow s milk cow s milk protein intolerance food intolerance

Adverse effects from foods are on record from ancient times Hippocrates observed that cow s milk could cause gastric upset and urticaria (4) but food hypersensitivity was rarely reported before von Pirquet introduced the concept of allergy in 1906 (32) Since 1966 the term allergy has been used mostly to describe IgE mediated hypersensitivity reactions (18 21) It is still not known how many hypersensitivity reactions to cow s milk proteins are mediated Therefore we prefer the term cow s milk protein intolerance meaning any adverse reaction whether of immediate or delayed type to cow s milk proteins giving symptoms in the gastrointestinal tract the skin or the respiratory tract

Estimates of the incidence of cow s milk protein intolerance in infants have produced considerably varying figures from 0 3% to 7 5% (1 5 6 13 15 31) This variation may be due to differences in the composition of the clinical materials to varying diagnostic criteria used for the diagnosis and also to a variation in the incidence from one area to another

This study determined the incidence of cow s milk protein intolerance in infancy in a Swedish urban community its various manifestations and the course of the disease during the first years of life

PATIENTS AND METHODS

Malmo a town in southern Sweden has about 250 000 inhabitants About 2 500 infants are born each year It has one obstetric and one children s hospital During the period of this study 45% of the infants in Malmö were entirely breastfed at 3 months and 0% at 6 months (L Righard M D Head of Child Welfare Clinics in Malmo personal communication) From 9 February to 24 March 1976 and 11 October 1976 to 17 April 1977 all women with a newborn infant at the obstetric hospital were informed by one of us (I J) about cow s milk protein intolerance They received both oral and written information about the design of the study and were invited to participate in it

During these periods 1555 infants were born alive 7 died neonatally Of the other 1548 the parents of 33 agreed to participate Four failed to fulfil the study (Fig 1) This group of 3 8 comprise *Group A* Of the other 1 16 751 (*Group B*) were followed up via records at child welfare clinics attended by physicians from the children s hospital or practitioner paediatricians The physicians and the nurses at the child welfare clinics were informed

4 Brostoff J Cellular hypersensitivity in milk allergy *Clin Allergy 1* 350 1970
5 Davidson G P Hill D J & Townley R R W Gastrointestinal milk allergy in childhood A rational approach *Med J Aust 1* 945 1976
6 Fontain J I & Navarro J Small intestinal biopsy in cow s milk protein allergy in infancy *Arch Dis Child 50* 357 1975
7 Freier S Paediatric gastrointestinal allergy *Clin Allergy 3* 597 1973
8 Goldman A S Anderson D W Jr Sellers W A Saperstein S Kniker W T Halpern S R & Collaborators Milk allergy 1 Oral challenge with milk and isolated milk proteins in allergic children *Pediatrics 32* 425 1963
9 Harries J Paediatric gastrointestinal allergy *In* J Brostoff (ed) *Clinical immunology allergy in paediatric medicine* Blackwell Oxford 1973 p 128
10 Heiner D C Sears J W & Kniker W T Multiple precipitins to cow s milk in chronic respiratory disease *Am J Dis Child 103* 634 1962
11 Huggins K G & Brostoff J Local production of specific IgE antibodies in allergic rhinitis patients with negative skin test *Lancet II* 148 1975
12 Jakobsson I & Lindberg T Cow s milk as a cause of infantile colic in breast fed infants *Lancet I* 437 1978
13 Johansson S G Bennich H & Berg T In vitro diagnosis of atopic allergy 3 Quantitative estimation of circulating IgE antibodies by the radio allergosorbent test *Int Arch Allergy 41* 443 1971
14 Kaufman H S Allergy in the newborn skin test reactions confirmed by the Prausnitz Kustner test at birth *Clin Allergy 1* 363 1971
15 Kjellman N Johansson S G O & Roth A Serum IgE levels in healthy children quantitated by a sandwich technique *Clin Allergy 6* 51 1976
16 Kuitunen P Visakorpi J K Savilahti E & Pelkonen P Malabsorption syndrome with cow s milk intolerance Clinical findings and course in 54 cases *Arch Dis Child 50* 351 1975
17 Mancini G Carbonaro A O & Heremans J F Immuno-chemical quantitation of antigens by single radial immunodiffusion *Immunochemistry* 2 235 1965
18 Matthew D J Taylor B Norman A P Turner M W & Soothill J F Prevention of eczema *Lancet I* 321 1977
19 Matthews T S & Soothill J F Complement activation after milk feeding in children with cow s milk allergy *Lancet II* 893 1970
20 Mueller H L Weiss R J O Leary D & Murray A B The incidence of milk sensitivity and the development of allergy in infants *N Engl J Med 268* 1220 1963
21 Parish W E Detection of reaginic and short term sensitizing anaphylactic or anaphylactoid antibodies to milk in sera of allergic and normal persons *Clin Allergy 1* 369 1971
22 Roy C & Dubois R Gastroenterologic disorders *In* R E Stiehm & V A Fulginiti (eds) *Immunologic disorders in infants and children* Saunders Philadelphia 1973 p 382
23 Shelton M J Meek F Goller I & Hosking C S Serum immunoglobulin levels in children *Aust J Med Technol 5* 113 1974
24 Shiner M Ballard J & Smith M E Small intestinal mucosa in cow s milk allergy *Lancet I* 136 1975
25 Sumithran E & Iyngkaran N Is jejunal biopsy really necessary in cow s milk protein intolerance? *Lancet II* 1122 1977
26 Townley R R W & Barnes G L Intestinal biopsy in childhood *Arch Dis Child 48* 480 1973
27 Townley R R W Khaw K T & Schwachman H Quantitative assay of disaccharidase activities of small intestinal mucosal biopsy specimens in infancy and childhood *Pediatrics 36* 911 1965
28 Walker W A & Isselbacher K J Intestinal antibodies *N Engl J Med 297* 767 1977
29 Wide L & Porath J Radioimmunoassay of proteins with the use of sephadex-coupled antibodies *Biochem Biophys Acta 130* 257 1966
30 Williams H E & Freeman M Milk inhalation pneumonia The significance of fat filled macrophages in tracheal secretions *Aust Paediat J 9* 286 1973
31 Wilson J F Lahey M E & Heiner D C Studies on iron metabolism V further observations on cow s milk induced gastrointestinal bleeding in infants with iron-deficiency anaemia *J Pediatr 84* 335 1974

Submitted Feb 6 1979
Accepted April 24 1979

(G L B) Department of Gastroenterology
Royal Children s Hospital
Melbourne
Australia 3052

Acta Pædiatr Scand 68 853–859 1979

A PROSPECTIVE STUDY OF COW S MILK PROTEIN INTOLERANCE IN SWEDISH INFANTS

I JAKOBSSON and T LINDBERG

From the Department of Pae liatrics Malmo General Hospit il Malmo S eden

ABSTRACT Jakobsson I and Lindberg T (Department of Paediatrics Malmo General Hosp tal Malmo Sweden) A prospective study of cow s milk protein intolerance in Swedish infants Acta Paediatr Scand 68 853 1979 —1 079 of 1 548 newborn infants were followed during their first year 328 were prospectively contacted once a month 751 were followed up at child welfare clinics Altogether 20 were diagnosed as being cow s milk protein intolerant (1 9%) Symptoms from the gastrointestinal tract and the skin predominated Only 2 had respiratory symptoms Ten had their symtpoms within one week after the introduction of cow s milk 3 of them at their first cow s milk-containing meal A further 4 already had symp toms when fed only human milk The others (6 infants) showed symptoms after more than one week on a cow s milk containing diet Before 2 years of age 13 had recovered Twelve of the cow s milk protein intolerant infants also showed adverse reactions to other foods soy protein intolerance being the most common (7 infants) A family history of allergy was found in 35% (116) of the 328 infants and in 70% (14) of those with cow s milk protein intolerance

KEY WORDS Cow s milk cow s milk protein intolerance food intolerance

Adverse effects from foods are on record from ancient times Hippocrates observed that cow s milk could cause gastric upset and urti caria (4) but food hypersensitivity was rarely reported before von Pirquet introduced the concept of allergy in 1906 (32) Since 1966 the term allergy has been used mostly to describe IgE mediated hypersensitivity reactions (18 21) It is still not known how many hypersensi tivity reactions to cow s milk proteins are mediated Therefore we prefer the term cow s milk protein intolerance meaning any adverse reaction whether of immediate or delayed type to cow s milk proteins giving symptoms in the gastrointestinal tract the skin or the respiratory tract

Estimates of the incidence of cow s milk protein intolerance in infants have produced considerably varying figures from 0 3% to 7 5% (1 5 6 13 15 31) This variation may be due to differences in the composition of the clinical materials to varying diagnostic crite ria used for the diagnosis and also to a vari tion in the incidence from one area to another

This study determined the incidence of cow s milk protein intolerance in infancy in a Swedish urban community its various mani festations and the course of the disease during the first years of life

PATIENTS AND METHODS

Malmo a town in southern Sweden has about 250 000 inhabitants About 2 500 infants are born each year It has one obstetric and one children s hospital During the period of this study 45% of the infants in Malmo were entirely breastfed at 3 months and 20% at 6 months (L Righard M D Head of Child Welfare Clinics in Malmo personal communication) From 9 February to 24 March 1976 and 11 October 1976 to 17 April 1977 all women with a newborn infant at the obstetric hospital were in formed by one of us (I J) about cow s milk protein in tolerance They received both oral and written informa tion about the design of the study and were invited to participate in it

During these periods 1 555 infants were born alive 7 died neonatally Of the other 1 548 the parents of 332 agreed to participate Four failed to fulfil the study (Fig 1) This group of 3 8 comprise G p A Of the other 1 16 751 (C o p B) were followed up via records at child welfare clinics attended by physicians from the children s hospital or practitioner paediatricians The physicians and the nurses at the child welfare clinics were informed

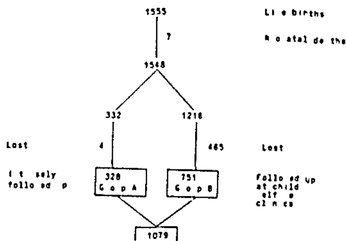


Fig 1 Survey of the number of infants

about the study Altogether 1 079 infants were followed up Of the other 465 (see Fig 1) one third had left the city the records of one third could not be found probably because of a change of surname and of one third because of moving several times from one district to another within the city

Follow up examinations

Group A Data were obtained from all parents concerning family history of allergy (eczema urticaria asthma hay fever) Once a month the parents gave on a standardized form data concerning each infant s height and weight (obtained at the child welfare clinics) and type of feeding Questions concerning problems were also answered (infantile colic diarrhoea vomiting bronchitis rhinitis exanthema) The parents could telephone any day to discuss problems

All infants were examined by one of us (I J) at age 3 6 and 12 months Those with symptoms that might be ascribed to allergy were examined more frequently

Group B Infants suspected of cow s milk protein intolerance were admitted to the children s hospital and examined by us We studied the records of the 751 infants at child welfare clinics with special regard to type of feeding gain in weight and height feeding difficulties infantile colic eczema or recurrent bronchitis during their first year of life Finally the hospital records of those infants admitted because of any problem were also studied

Managements of infants suspected of cow s milk protein intolerance

Cow s milk protein intolerance was suspected in any infant with one or more of the following symptoms vomiting and/or diarrhoea not due to any other demonstrable cause infantile colic not disappearing after advice on feeding technique or treatment with dimethylpolysiloxane (Minifom®) eczema urticaria and exanthemas without known relation to other foods and recurrent bronchitis

The infant was given a diet free from cow s milk—if possible human milk otherwise a soy based formula Those not tolerating soy protein were given a formula based on casein hydrolysate (Nutramigen® Mead Johnson) Infants with symptoms when fed human milk initially continued at the breast with the mother on a cow s milk free diet

If the symptoms disappeared on a cow s milk protein free diet the infant was challenged with whole cow s milk The challenges were performed after a period of cow s milk withdrawal varying from 4 to 70 weeks (mean 8 4 weeks) (In one infant it was performed after 7 weeks with a negative result) Gradually increasing amounts of whole cow s milk were given every third hour beginning with 1 ml and finishing with 50 ml The challenge was completed when a distinct positive reaction was seen At a suspected positive reaction the last dose was repeated once A symptom free infant had a total of 176 ml cow s milk

We made no challenges to diagnose food intolerance other than cow s milk protein intolerance However the parents spontaneously often performed challenges and the reactions were carefully recorded

Diagnostic criteria

The diagnosis of cow s milk protein intolerance was based on (*a*) the presence of symptoms on a diet containing cow s milk (*b*) the disappearance of symptoms on withdrawal of cow s milk (*c*) at least two positive milk challenges with return of identical symptoms

Lactose intolerance was excluded by a careful history by the infant tolerating human milk and in doubtful cases by a blood glucose rise of ≥1 1 mmol/l and no symptoms following a lactose tolerance test (dose 2 g/kg body weight)

RESULTS

Incidence

A total of 20 infants (11 boys and 9 girls) fulfilled the criteria given above and were diagnosed as cow s milk protein intolerant (Table 1) Five reacted within one hour after challenge and six after more than 24 h (24–96 h) Cow s milk proteins were eliminated from the diet in a further 30 because of suspected symp-

Table 1 *Incidence of cow s milk protein intolerance*

For explanation of groups A and B see Fig 1

	No of infants	%
Group A (*n*=328)	12	3 7
Group B (n=751)	*8*	*1 1*
Total (*n*=1 079)	20	1 9

Table 2 *Symptomatology and course of the disease in 20 infants with cow s milk protein intolerance*

Patient	Introduction of cow s milk age (weeks)	Onset of symptoms age (weeks)	Elimination of cow s milk age (weeks)	Symptoms	Positive milk challenges age (weeks)	Tolerant to cow s milk age (weeks)	Intolerances to other foods
FA ♂	0	20	28	*D* B E	32 60 96	>104	Orange tomato soy protein
PS ♂	8	44	51	E	72 104	>104	Orange egg fish
VL ♀	16	3	3	*C* D V Ex E	8 24 72	>104	Wheat soy protein
FT ♂	Never	6	16	E	20 40 72	>104	Orange egg wheat nuts soy protein
AS ♀	10	24	24	*C* V U Ex E	28 52	>104	Fish soy protein
CA ♀*	16	16	20	V E	32 40 72 104	>104	Orange tomato
TM ♀	24	24	44	D	60 80	>104	–
MC ♂	18	18	24	V *U* Ex E	40 48	>104	Egg soy protein
AN ♂	0	4	4	Ex	8 1	20	–
DL ♂	1/2	26	28	U Ex	36 40	48	Several fruit juices
EI ♀	16	16	16	V *Ex D*	20 28	44	–
MH ♂	3	20	28	E	32 40	48	Orange rosehip egg fish
AK ♀	8	4	4	*C* V	8 16	28	–
TI [illegible]	5	6	12	C V	16 24	36	Rosehip soy protein
MD ♂*	8	8	8	V	16 40	60	–
ME ♂		[illegible]	9	*V W*	12 16	40	Soy protein
AR ♂	3	3	9	*C V D* W	16 20	28	–
PH ♀	2	4	4	*C V*	3 40	48	–
DS ♀	8	8	15	C U Ex	20 40	56	–
DE ♂	2		8	*E* B	12 24	5	Orange

V=vomiting D=diarrhoea C=infantile colic U=urticaria Ex=exanthema E=eczema B=bronchitis W=slow weight gain Italics=first symptom(s)

* Symptoms at first cow s milk containing meal

First symptoms when fed only human milk

toms In 7 the elimination had *no effect* *In* 23 the symptoms disappeared and did not return when the infants were challenged with cow s milk They continued on a cow s milk based formula without *any symptoms* They were followed for at least 2 *months* after the challenge 12 up to one year of age

Symptoms

Table 2 presents symptoms and course *of the* disease in the infants with cow s *milk protein* intolerance Seven infants had *symptoms from* the gastrointestinal tract only in 2 combined with a slow *weight gain* One had diarrhoea as the predominant *symptom* a 6-month old girl tolerating human milk got diarrhoea and abdominal pain on cow s milk on several challenges A lactose tolerance test was normal *She had* normal stools on a cow s milk free diet and *no signs of intestinal infection* Secondary lactose intolerance could thus be excluded Seven infants had infantile colic In 3 of them colic was the only symptom at the debut but was ignored and the infants were not admitted until other symptoms occurred Five infants presented symptoms from the skin only and 6 from both the skin and gastro*intestinal* tract Two had symptoms from the respiratory tract but they also reacted from *the skin* and intestinal tract Four of the infants had symptoms when fed only *human milk* The symptoms disappeared with the *mothers* on a cow s milk free diet and returned *with the* mother on a normal diet Identical *symptoms* appeared on direct challenge with whole cow s milk

Table 3 *Onset of symptoms in relation to the introduction of cow s milk in infants with cow s milk protein intolerance*

	No of infants
<1 week	10[a]
1–4 weeks	2
>4 weeks	4

Four infants had their first symptoms when fed only human milk

[a] Three had their first symptoms at first cow s milk containing meal

All 5 infants reacting within one hour after challenge vomited 2 also had urticaria and exanthema one colic and diarrhoea Of the 6 infants reacting after more than 24 h after challenge 3 had eczema 1 diarrhoea 2 diarrhoea combined with colic and vomiting

Onset of symptoms

Eleven infants had their first symptoms before 8 weeks 8 between 12 and 26 weeks and one boy at 44 weeks of age (Table 2) The latter had been fed cow s milk since 8 weeks of age but got cow s milk dependent eczema at 44 weeks of age Infantile colic was the predominant symptom in the infants reacting before 8 weeks of age (6/11)

Table 3 shows that 10 infants got their symptoms with one week after the introduction of cow s milk Three reacted on their first cow s milk containing meal

Infants who became cow s milk protein intolerant were entirely breastfed as long as the other infants (13 weeks v 12 weeks (mean value))

Duration of the intolerance

Nine infants became cow s milk tolerant before 1 year of age 3 of them before 6 months (Table 2) At 2 years of age a further 3 had recovered Eight infants aged 2–3 years are still intolerant to cow s milk 7 of them have multiple food intolerances and eczema

Associated food intolerances

Twelve infants with cow s milk protein intolerance also showed intolerance to other foods (soy egg fish wheat tomato orange nuts) Seven were intolerant to soy protein which was the most common food intolerance associated with cow s milk protein intolerance Ten infants had adverse reactions to several types of foods (see Table 2)

Intolerances to foods other than cow s milk

Adverse reactions to foods other than cow s milk (e g egg fish wheat tomato orange strawberry) were reported in 26% (85) of the Group A infants Many of these reactions were diffuse exanthemas after eating some kind of fruit The reactions had occurred at least twice before we considered them as food dependent These intolerance periods were usually brief— a few months

Allergic heredity

A family history of allergy (mother father and/or sibling) was found in 35% (116) of the 328 infants in Group A In the 20 infants with milk protein intolerance the corresponding figure was 70% (14)

DISCUSSION

The diagnosis of cow s milk intolerance is still clinically based so far there is no laboratory test which can distinguish one individual with this condition from others (3 7 8 10 12 19 26 28)

The diagnosis must therefore be based on clinical observation of the effects of elimination and introduction of cow s milk The original criteria for making the diagnosis of cow s milk protein intolerance according to Goldman (14) with three positive milk challenges is often impossible to fulfil It is often unacceptable to make repeated milk challenges at short intervals This procedure also probably leads to fewer diagnoses of the disorder as the

infants can have become tolerant before the third challenge Therefore most clinicians now would accept one or two positive challenges as strong evidence in favour of the diagnosis (33) we used two positive challenges as a diagnostic criterion

It is important to exclude lactose intolerance as a cause of the infant s symptoms Mostly this is easily done by a careful history In only one infant in our study had a lactose tolerance test to be made The results of lactose tolerance tests could be of value but must be interpreted with caution as up to 25% might be false positives (2 17 23) It is also evident that lactose intolerance might accompany cow s milk protein intolerance (16 25) Moreover it has been reported that commercial lactose preparations might contain small amounts of cow s milk proteins (30)

Previous studies on the incidence of cow s milk protein intolerance gave figures varying from 0 3 to 7 5% The incidence 0 3% is given by Collins Williams (6) after excluding all patients with major allergies and does not represent a normal population A retrospective study by Bachman & Dees (1) found that 1% of 403 infants had cow s milk protein intolerance Recently Stintzing & Zetterstrom retrospectively calculated an incidence of 0 58% (31) The incidence in two other prospectively studied groups of infants is reported to be 1 5% (15) and 7 5% (13) The overall incidence in our study was 1 9% but in the group most intensely studied (Group A) it was 3 7% Naturally parents with allergy problems of their own are more interested in taking part in a study such as this Therefore there is probably an inevitable selection in Group A with a 35% family history of allergy but this figure does not differ much from that reported by Kjellman (30%) in a Swedish unselected population (22) Moreover Group A probably includes infants with mild forms of cow s milk protein intolerance not otherwise diagnosed

The symptomatology at the debut agrees with previous studies (1 5 6 11 13) Gerrard et al (13) found that about 40% of the infants with cow s milk protein intolerance developed their symptoms within one week after their first exposure to cow s milk In our study the corresponding figure is 14/20 (70%) Four had symptoms already when fed only human milk and three at their first cow s milk containing meal This supports the hypothesis that cow s milk protein sensitization can occur *in utero* (27) or via human milk (9 20 29) A further 4 infants of Group A with severe infantile colic when fed only human milk were free of symptoms when their mothers had a cow s milk free diet (20) They were challenged several times with positive results by giving the mothers a normal diet However when challenged directly with whole cow s milk at age 3–4 months they had no symptoms and thus did not fulfil our diagnostic criteria

The frequency of soy protein intolerance in cow s milk protein intolerant infants in our study (35%) agrees with other reports (11) but is higher than that of Kuitunen et al (24) who studied infants with malabsorption syndrome because of cow s milk intolerance

26% of Group A had adverse reactions to foods other than cow s milk Of the infants with cow s milk protein intolerance the corresponding figure was 60% It is important to bear in mind this increased tendency towards associated food intolerances also reported by others (11 13 24) when introducing new foods in the diet for the cow s milk protein intolerant infant

Gerrard et al (13) found that 27% had lost their sensitivity to cow s milk before 2 years of age Clein (5) reports as many as 95% before 2 years Our corresponding figure is 60% The future prospects of those who are cow s milk protein intolerant in infancy are obscure No infant of our study has so far been followed for more than 3 years According to Clein (5) 80% of infants intolerant to cow s milk develop other allergic manifestations before they reach the age of puberty

In conclusion the incidence of cow s milk protein intolerance in infancy in a Swedish urban community was 20 out of c 1000 In 14

of the 20, the onset of symptoms occurred within one week after first exposure to cow's milk. Thirteen of the 20 recovered from the intolerance before 2 years of age.

ACKNOWLEDGEMENTS

Grant from The Swedish Medical Research Council (project no. 5143 and 5364), Albert Påhlssons Foundation, Alfred Österlund Foundation, The Medical Research Council of the Swedish life insurance companies, The Swedish Baby Food Industry Fund for Nutritional Research and Swedish Nutrition Foundation supported this work.

Mrs I. Nilsson gave valuable assistance.

REFERENCES

1. Bachman, K. D. & Dees, S. C.: Milk allergy. I. Observations on incidence and symptoms in "well" babies. Pediatrics 20: 393, 1959.
2. Berg, N. O., Borulf, S., Jakobsson, I. & Lindberg, T.: How to approach the child suspected of malabsorption. Acta Paediatr Scand 67: 403, 1978.
3. Berg, N. O., Jakobsson, I. & Lindberg, T.: Do pre- and postchallenge small intestinal biopsies help to diagnose cow's milk protein intolerance? Acta Paediatr Scand 68: 657, 1979.
4. Chabot, R.: Pediatric Allergy. New York: McGraw-Hill Book Co. Inc., 1951.
5. Clein, N. W.: Cow's milk allergy in infants and children. Int Arch Allergy 13: 245, 1958.
6. Collins-Williams, C.: The incidence of milk allergy in pediatric practice. J Pediatr 48: 39, 1956.
7. Dannaeus, A., Johansson, S. G. O., Foucard, T. & Öhman, S.: Clinical and immunological aspects of food allergy in childhood. I. Estimation of IgG, IgA and IgE antibodies to food antigens in children with food allergy and atopic dermatitis. Acta Paediatr Scand 66: 31, 1977.
8. Dannaeus, A.: Food allergy in childhood. A study of the humoral immune response to basic food antigens in relation to clinical symptoms. Thesis. Acta Univ Upsal 312, 1979.
9. Donnally, H. H.: The question of the elimination of foreign protein (egg white) in woman's milk. J Immunol 19: 15, 1930.
10. Editorial: Cow's milk protein and the small intestine. Lancet I: 702, 1978.
11. Freier, S.: Paediatric gastrointestinal allergy. Clin Allergy 3: 597, 1973.
12. Fällström, S. P., Ahlstedt, S. & Hanson, L. Å.: Specific antibodies in infants with gastrointestinal intolerance to cow's milk protein. Int Arch Allergy Appl Immunol 56: 97, 1978.
13. Gerrard, J. W., Mackenzie, J. W. A., Goluboff, N., Garson, J. Z. & Maningas, C. S.: Cow's milk allergy: Prevalence and manifestations in an unselected series of newborns. Acta Paediatr Scand Suppl 234, 1973.
14. Goldman, A. S., Anderson, D. W. Jr, Sellers, W. A., Saperstein, S., Kniker, W. T. & Halpern, S. R.: Milk allergy. I. Oral challenge with milk and isolated milk proteins in allergic children. Pediatrics 32: 425, 1963.
15. Halpern, S. R., Sellars, W. A., Johnson, R. B., Anderson, D. W., Saperstein, S. & Reisch, J. S.: Development of childhood allergy in infants fed breast, soy or cow's milk. J Allergy Clin Immunol 51: 139, 1973.
16. Harrison, M., Kilby, A., Walker-Smith, J. A., France, N. E. & Wood, C. B. S.: Cow's milk protein intolerance: a possible association with gastroenteritis, lactose intolerance and IgA deficiency. Br Med J I: 1501, 1976.
17. Harrison, B. M. & Walker-Smith, J. A.: Reinvestigation of lactose intolerant children: lack of correlation between continuing lactose intolerance and small intestinal morphology, disaccharidase activity and lactose tolerance tests. Gut 18: 48, 1977.
18. Ishizaka, K., Ishizaka, T. & Hornbrook, M. M.: Physiochemical properties of human reaginic antibody. IV. Presence of a unique immunoglobulin as a carrier of reaginic activity. J Immunol 97: 75, 1966.
19. Iyngkaran, N., Robinson, M. J., Sumithran, S. K., Lam, S. K., Puthucheary, S. D. & Yadav, M.: Cow's milk protein sensitive enteropathy. An important factor in prolonging diarrhoea of acute infective enteritis in early infancy. Arch Dis Child 53: 150, 1978.
20. Jakobsson, I. & Lindberg, T.: Cow's milk as a cause of infantile colic in breastfed infants. Lancet II: 437, 1978.
21. Johansson, S. G. O., Bennich, H. & Wide, L.: A new class of immunoglobulin in human serum. Immunology 14: 265, 1968.
22. Kjellman, N. I. M.: Immunoglobulin E and atopic allergy in childhood. Linköping University Medical Dissertations No. 36, 1976.
23. Krasilnikoff, P. A., Gudmund-Høyer, E. & Moltke, H. H.: Diagnostic value of disaccharide tolerance tests in children. Acta Paediatr Scand 64: 693, 1975.
24. Kuitunen, P., Visakorpi, J. K., Savilahti, E. & Pelkonen, P.: Malabsorption syndrome with cow's milk intolerance. Clinical findings and course in 54 cases. Arch Dis Child 50: 351, 1975.
25. Lin, H. Y., Tsao, M. U., Moore, B. & Giday, Z.: Bovine milk protein—induced intestinal malabsorption of lactose and fats in infants. Gastroenterology 54: 27, 1967.
26. Matthews, T. S. & Soothill, J. F.: Complement activation after milk feeding in children with cow's milk allergy. Lancet II: 893, 1970.
27. Matsumura, T., Kuroume, T., Oguri, M., Iwasaki, I., Kanbe, Y., Yamada, T., Kawabe, S. & Negishi, K.: Egg sensitivity and eczematous manifestations in breastfed newborns with particular reference to intrauterine sensitization. Ann Allergy 35: 222, 1975.
28. Savilahti, E.: Immunochemical study of the malabsorption syndrome with cow's milk intolerance. Gut 14: 4[illegible], 19[illegible].

29 Shannon W R Demonstration of food proteins in human breast milk by anaphylactic experiments on guinea pigs *Am J Dis Child* 22 223 1921
30 Spies J R New antigens in lactose *Proc Soc Exp Biol Med 137* 211 1971
31 Stintzing G & Zetterstrom R Cow s milk allergy incidence and pathogenetic role of early exposure to cow s milk formula *Acta Paediatr Scand 68* 383 1979
32 Von Pirquet C Allergie *Munch Med Wochenschr* 53 1457 1906
33 Walker Smith J Cow s milk protein intolerance Transient food intolerance of infancy *Arch Dis Child 50* 347 1975

Submitted April 3 1979
Accepted June 6 1979

(I J) Department of Paediatrics
Malmo General Hospital
S 21401 Malmo
Sweden

of the 20 the onset of symptoms occurred within one week after first exposure to cow s milk Thirteen of the 20 recovered from the intolerance before 2 years of age

ACKNOWLEDGEMENTS

Grant from The Swedish Medical Research Council (project no 5143 and 5364) Albert Påhlssons Foundation Alfred Österlund Foundation The Medical Research Council of the Swedish life insurance companies The Swedish Baby Food Industry Fund for Nutritional Research and Swedish Nutrition Foundation supported this work

Mrs I Nilsson gave valuable assistance

REFERENCES

1 Bachman K D & Dees S C Milk allergy I Observations on incidence and symtoms in well babies *Pediatrics 20* 393 1959
2 Berg N O Borulf S Jakobsson I & Lindberg T *How to approach the child suspected of malabsorption Acta Paediatr Scand 67* 403 1978
3 Berg N O Jakobsson I & Lindberg T Do pre and postchallenge small intestinal biopsies help to diagnose cow s milk protein intolerance? *Acta Paediatr Scand 68* 657 1979
4 Chabot R *Pediatric Allergy* New York McGraw Hill Book Co Inc 1951
5 Clein N W Cow s milk allergy in infants and children *Int Arch Allergy* 13 245 1958
6 *Collins Williams C The incidence of milk allergy in* pediatric practice *J Pediatr 48* 39 1956
7 Dannaeus A Johansson S G O Foucard T & Öhman S Clinical and immunological aspects of food allergy in childhood I Estimation of IgG IgA and IgE antibodies to food antigens in children with food allergy and atopic dermatitis *Acta Paediatr Scand* 66 31 1977
8 Dannaeus A Food allergy in childhood A study of the humoral immune response to basic food antigens *in relation* to clinical symptoms Thesis *Acta Univ Upsal 312* 1979
9 Donnally H H The question of the elimination of foreign protein (egg white) in woman s milk *J Immunol 19* 15 1930
10 Editorial Cow s milk protein and the small intestine *Lancet I* 702 1978
11 Freier S Paediatric gastrointestinal allergy *Clin Allergy 3* 597 1973
12 Fallstrom S P Ahlstedt S & Hanson L Å Specific antibodies in infants with gastrointestinal intolerance to cow s milk protein *Int Arch Allergy Appl Immunol 56* 97 1978
13 Gerrard J W Mackenzie J W A Goluboff N Garson J Z & Maningas *C S Cow s* milk allergy Prevalence and manifestations in an unselected series of newborns *Acta Paediatr Scand* Suppl 234 1973
14 Goldman A S Anderson D W Jr Sellers W A Saperstein S Kniker W T & Halpern S R Milk allergy I Oral challenge with milk and isolated milk proteins in allergic children *Pediatrics 3?* 425 1963
15 Halpern S R Sellars W A Johnson R B *Anderson D W Saperstein S & Reisch J S* Development of childhood allergy in infants fed breast soy or cow s milk *J Allergy Clin Immunol 51* 139 1973
16 Harrison M Kilby A Walker Smith J A France N E & Wood C B S Cow s milk protein intolerance a possible association with gastroenteritis lactose intolerance and IgA deficiency *Br Med J I* 1501 1976
17 Harrison B M & Walker Smith J A Reinvestigation of lactose intolerant children lack of correlation between continuing lactose intolerance and small intestinal morphology disaccharidase activity and *lactose tolerance tests Gut 18* 48 1977
18 Ishizaka K Ishizaka T & Hornbrook M M Physiochemical properties of human reaginic antibody IV Presence of a unique immunoglobulin as a carrier of reaginic activity *J Immunol 97* 75 1966
19 Iyngkaran N Robinson M J Sumithran S K Lam S K Puzhucheary S D & Yadar M Cow milk protein sensitive enteropathy *An important* factor in prolonging diarrhoea of acute infective enteritis in early infancy *Arch Dis Child 53* 150 1978
20 Jakobsson I & Lindberg T Cow s milk as a cause of infantile colic in breastfed infants *Lancet II* 437 1978
21 Johansson S G O Bennich H & Wide L A new class of immunoglobulin in human serum *Immunology 14* 265 1968
22 Kjellman N I M *Immunoglobulin* E and atopic allergy in childhood *Linkoping University Medical Dissertations* No *36* 1976
23 Krasilnikoff P A Gudmund Hoyer E & Moltke H H Diagnostic value of disaccharide tolerance tests in children *Acta Paediatr Scand 64* 693 1975
24 Kuitunen P Visakorpi J K Savilahti E & Pelkonen P *Malabsorption syndrome with cow s milk* intolerance Clinical findings and course in 54 cases *Arch Dis Child 50* 351 1975
25 Lin H Y Tsao M U Moore B & Giday Z Bovine milk protein—induced intestinal malabsorption of lactose and fats in infants *Gastroenterology 54* 27 1967
26 *Matthews T S & Soothill J F Complement* activation after milk feeding in children with cow milk allergy *Lancet II* 893 1970
27 Matsumura T Kuroume T Oguri M Iwasaki I Kanbe Y Yamada T Kawabe S & Negishi K Egg sensitivity and eczematous manifestations in breastfed newborns with particular reference to in *trauterine sensitization Ann Allergy 35* 2?? 1975
28 Savilahti E Immunochemical study of the malabsorption syndrome with cow s milk intolerance

9 Shannon W R Demonstration of food proteins in human breast milk by anaphylactic experiments on guinea pigs *Am J Dis Child* 22 223 1921
0 Spies J R New antigens in lactose *Proc Soc Exp Biol Med* 137 211 1971
1 Stintzing G & Zetterstrom R Cow s milk allergy incidence and pathogenetic role of early exposure to cow s milk formula *Acta Paediatr Scand* 68 383 1979
Von Pirquet C Allergie *Munch Med Wochenschr* 53 1457 1906
33 Walker Smith J Cow s milk protein intolerance Transient food intolerance of infancy *Arch Dis Child* 50 347 1975

Submitted April 3 1979
Accepted June 6 1979

(I J) Department of Paediatrics
Malmo General Hospital
S 214 01 Malmö
Sweden

Acta Pædiatr Scand 68 861–868 1979

CONGENITAL PERSISTENT PROXIMAL TYPE RENAL TUBULAR ACIDOSIS IN TWO BROTHERS'

ARNT WINSNES EIRIK MONN ODDVAR STOKKE and TOR FEYLING

From the Department of Paediatrics and Clinical Chemistry Rikshospitalet Oslo and the Department of Paediatrics Fylkessjukehuset i Kristiansund Kristiansund Norway

ABSTRACT Winsnes A Monn E Stokke O and Feyling T (Depts of Paediatrics and Clinical Chemistry Rikshospitalet Oslo and Dept of Paediatrics Fylkessjukehuset i Kristiansund Kristiansund Norway) Congenital persistent proximal type renal tubular acidosis in two brothers Acta Paediatr Scand 68 861 1979 —Two brothers showed severe and persistent hyperchloraemic metabolic acidosis (capillary blood pH 7 07–7 15) due to a low renal bicarbonate threshold at 11 mmol/l The maximal tubular capacity for bicarbonate reabsorption was reduced to about half the normal A high dose of acetazolamide (25 mg/kg) lowered the tubular bicarbonate reabsorption substantially indicating the presence of carbonic anhydrase Both the glomerular filtration rate the renal blood flow and the renal concentrating capacity were slightly reduced The clinical characteristics were growth retardation mental retardation nystagmus corneal opacities cataract glaucoma and enamel defects of the permanent teeth Serum thyroxine was pathologically low without clinical signs of hypothyreosis The erythrocytes showed an increased osmotic resistance Autopsy of the younger brother who died 4 ½ years old revealed thyroid and thymus weights of 25% of the normal The kidney tubular cells were swollen with vacuoles The glomeruli had a normal appearance

KEY WORDS Renal tubular acidosis bicarbonate wasting growth retardation mental retardation cataract glaucoma nystagmus

At least two physiologically distinct tubular disorders may give rise to renal tubular acidosis (RTA) (18 19) The classical or distal RTA is characterized by an inability of the distal tubule to generate a sufficiently large hydrogen ion gradient between blood and the tubular fluid (7 8 10 17 20) Thus the excretion of ammonium ions and titratable acid are reduced and urinary pH is usually above 6 5 despite overt acidosis In the proximal or bicarbonate wasting type of RTA on the other hand excretion of acid in the distal tubules is normal and the urine is normally acidic with a pH down to 5 during acidosis In this type of RTA an impaired ability to reabsorb bicarbonate in the proximal tubules causes the hyperchloraemic acidosis (18 19)

In the majority of cases of proximal RTA multiple dysfunctions of the proximal tubules are present (Fanconi syndrome) and the renal disorder is secondary to a systemic disease i e galactosaemia tyrosinaemia cystinosis etc (8 10 20) The so called primary form of proximal RTA where no systemic disease can be demonstrated and where no tubular dysfunction except bicarbonate wasting is present usually appears as a transitory defect in male infants with growth failure as the main clinical feature (11)

A primary familial form of persistent proximal RTA was recently described (1) The affected family members were however asymptomatic and the only clinical finding was short stature (1) The purpose of this article is to report the unusual clinical characteristics of another form of proximal RTA that is persistent hereditary and apparently primary

---

' A short presentation of this condition was given at the Tenth Annual Meeting of the European Society for Paediatric Nephrology in Barcelona 1976

METHODS AND PROCEDURES

Renal tubular function studies were performed on the patient B H at the age of 10 years Bicarbonate reabsorption was studied by infusion of sodium bicarbonate (250 mmol/l) at rates gradually increasing from 25 to 75 mmol/hour Inulin was infused from a separate flask for the determination of GFR

Urine was collected from an urethral catheter at 30 min intervals and the pH was measured immediately at 37° Blood samples were obtained from an indwelling venous cannula at midpoint during each period of urine collection

Titratable acid in urine is the amount of base equivalents necessary for adjustment to the actual pH of the blood Clinical chemical data were obtained with conventional methods

CASE REPORTS

The family

The family consists of the unrelated parents and three boys B H O H and S H With the exception of B H and O H there is no history of mental or growth retardation and no occurrence of acidosis nystagmus cataract or glaucoma The father was born in 1945 His height is 181 cm The mother was born in 1944 Her height is 167 cm She her two sisters and their parents have diabetes mellitus

Clinical histories of the patients B H and O H

B H and O H were born after normal pregnancies at term in 1966 and 1968 respectively with birth weights of 4 050 and 3 670 g and length 51 cm They could both walk at 18 months The growth of B H and O H was retarded during the first year but later their heights have been at −2 S D The speech development of both B H and O H has been retarded At the age of 12 years B H can speak incomplete sentences with 4–5 words He has learned some of the letters of the alphabet but cannot read

The severe metabolic acidosis was diagnosed at 5 and 3½ years of age respectively and has been unchanged later

Both patients have experienced several episodes of inappropriately severe symptoms with sopor and vomiting after slight to moderate head traumas The degree of acidosis has been unchanged on these occasions O H died 4½ years old during such an episode lasting three days after a moderate head trauma At autopsy of the brain there was fresh bleeding in the corpus callosum and in the right temporal lobe

Both patients had undulating nystagmus from the neonatal period and both had slight milky white opacities in the cornea when first examined by an ophthalmologist at 5 and 3½ years of age Cataract in the rear part of the lens has been noted in B H since he was 9 years A steady progress leading to severely reduced vision will necessitate enucleation in the near future At eleven years bilateral glaucoma was diagnosed immediately operated and later treated with pilocarpine and timolol eye drops

Laboratory data for B H and O H The range of data are from both patients whereas mean values are given in parenthesis for B H and O H respectively Capillary blood pH 7 07–7 15 (7 12 7 11) standard bicarbonate 7–11 (10 9) mmol/l pCO 2 9–4 3 (3 5 3 9) kPa *Serum concentrations* in mmol/l Na 135–140 (138 138) Cl 118–128 (122 120) K 3 2–4 1 (3 7 3 3) Ca 2 1–2 3 (2 2 2 2) P 1 5–1 7 (1 6 1 6) urea 5 7–8 4 (7 5 6 2) and creatinine 44–71 (53 71) μmol/l

Haematological parameters in B H and O H respectively at the time when the osmotic fragility of erythrocytes was measured Hgb 13 1 and 10 4 g/100 ml Hct 42 and 35% MCV 90 and 99 fl MCH 28 and 29 5 pg MCHC 31 and 30 g/100 ml reticulocytes 5 5 and 7 4% thrombocytes 123 and 172×10 /l

Serum concentrations measured at least once with normal results in B H Mg Zn Pb uric acid lactate pyruvate amino acids bilirubin haptoglobin folic acid iron total iron binding capacity vitamin B_{12} alkaline phosphatases transaminases lactic dehydrogenase creatine kinase total cholesterol triglycerides phospholipids

Hormones in B H Parathormone calcitonin and growth hormone were normal Aldosterone in serum was either normal or moderately increased whereas aldosterone excretion in the urine was normal Plasma insulin values were 30 and 49 μU/ml despite normal blood glucose and lack of obesity

Thyroid function Thyroxin values in B H were 32 39 and 45 nmol/l at 5 9 and 10 years and in O H at 4 years thyroxin was 54 nmol/l despite treatment with l Thyroxin Free thyroxine index and protein bound iodine were likewise pathologically low in both patients whereas tri iodothyronine TSH and thyroxine binding globulin were normal

Erythrocyte enzymes in B H Analyses of 8 different erythrocyte enzymes associated with haemolytic anaemias revealed activities higher than normal possibly due to a somewhat younger mean cell age The total carbonic anhydrase activity was normal The lipid composition of the erythrocyte membranes was also normal as was the scanning electron microscopic appearance

Sweat electrolytes in B H were normal except for an increased chloride concentration at 80 mmol/l

Gastric acid production increased normally upon stimulation with histamine

Urine analyses The pH was around 5 protein amino acids lysozyme citrate and calcium were excreted in normal amounts Cyclic AMP excretion increased normally upon stimulation with parathormone Glucosuria occurred only after increasing the blood glucose level to above 14 mmol/l Alpha ketoglutarate was usually excreted in large amounts up to 300 mg/day in the urine of both patients Otherwise the gas chromatograms of the organic acids in urine were normal

Autopsy findings in O H The thyroid gland was smaller than normal 1 g as against 4 g normal weight at this age The microscopic examination of the thyroid gland showed a somewhat reduced follicle epithelium but no obvious pathological findings The thymus weight was also low about 25% of normal but without significant microscopic changes In the kidneys the glomeruli appeared normal In the proximal tubular epithelium cells vacuolization and increased fat content was observed

Table 1 *Renal function parameters of B H*

Test	Patient	Normal
Creatinine clearance (ml/min) (1 73 m)	47 68 70 84	70–130
Inulin clearance (ml/min) (1 73 m²)	55 61 61	90–150
Effective renal plasma flow (J Hippuran) (ml/min) (1 73 m²)	364	450–650
Tubular reabsorption of phosphate	70 7? 83	80–95
Titratable acid (mmol/min) (1 73 m)	17 ?1 25 31	4–16
Ammonium excretion (μmol/min) 1 73 m²)	8 9 1 ?2	6–?6
Urine pH	4 9–5 1	
Diuresis ml/?4 hours	800 – 1 000	
Concentrating capacity mosmol/kg water deprivation + Vasopressin	740 – 780	(814–1 3?4)

RESULTS AND DISCUSSION

Acid base and electrolyte metabolism

B H and O H have exhibited a marked hyperchloraemic metabolic acidosis that in the case of B H has been unchanged during an observation period of 7 years Urinary pH has constantly been around 5 and excretion of both titratable acid and ammonium were in the normal range in B H (Table 1)

During bicarbonate infusion the urinary pH increased immediately when the serum level of bicarbonate started to increase (Fig 1) No bicarbonate was found in the urine in the basal condition whereas large amounts were excreted at a normal serum bicarbonate level (Fig 2) increasing the urinary pH to around 8 (Fig 1)

Our patients thus clearly belong to the proximal or bicarbonate wasting category of RTA The bicarbonate threshold of B H was found to be 11 mmol/l (Figs 1 & 2) which is considerably lower than that reported by Soriano and coworkers (18) in primary proximal RTA where thresholds in the range 18–20 mmol/l were found However the 20-months old girl described by Donckerwolcke et al (2) had a bicarbonate threshold expressed as total CO_2 in serum of 10 3 mmol/l which was similar to that of B H

The tubular capacity for reabsorption of bicarbonate was essentially unchanged at serum bicarbonate levels between 14 and 26 mmol/l (Fig 2) The maximal tubular transport capacity for bicarbonate (Tm–HCO_3) was 1 29 1 13 and 1 14 mmol per 100 ml of glomerular filtrate (G F) in the bicarbonate infusion studies this being similar to the girl reported by the Dutch group (2) but again much lower than that reported in the American patients (18)

The Tm–HCO_3 values observed may be lower than the true capacity since expansion of the extracellular volume which is unavoidable during bicarbonate infusion will lower bicarbonate reabsorption (12) However this error will be of similar magnitude in the clinical studies reported (2 18) the differences observed thus being significant In addition the theoretical error in Tm–HCO_3 is probably of minor importance since the total

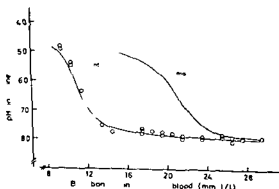


Fig 1 Relationship of urinary pH to actual bicarbonate in venous blood in B H during $NaHCO_3$ infusion The normal curve (drawn by inspection) represents the mean response of healthy infants as described by Soriano et al (18)

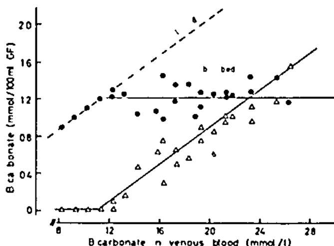


Fig. 2. Relation between venous blood concentration, renal tubular reabsorption and urinary excretion of bicarbonate in B. H. The stippled line represents the theoretical line of 100% reabsorption. Filled symbols represent reabsorbed and open symbols excreted bicarbonate.

amount of fluid given during the bicarbonate infusion studies in B. H. was similar to the volume of urine voided (1 500–1 800 ml). Furthermore, the serum creatinine concentration remained remarkably constant, 53–49–51 mmol/l during the bicarbonate infusion. Serum Na increased from 136 to 145 mmol/l.

The bicarbonate infusion normalized both serum bicarbonate and chloride, whereas serum potassium decreased rapidly from 3.5 to a dangerously low level at 2 mmol/l. This precipitous serum K fall is probably due to an increased Na–K exchange within the distal tubules presented with great amounts of $NaHCO_3$. Bicarbonate administration is known to increase the serum level of parathormone leading to phosphaturia (5), possibly explaining the development of pronounced hypophosphataemia with a fall from 2.1 to 0.23 mmol/l.

In healthy individuals with normal bicarbonate levels, the proximal tubules reclaim 85–90% of the filtered bicarbonate. The enzyme carbonic anhydrase present both in the brush border and the interior of the proximal tubular cells is thought to be responsible for most of this reabsorption (7, 17). Thus, when the carbonic anhydrase inhibitor acetazolamide is given, an increased bicarbonate excretion in urine normally occurs. Such a response represents indirect evidence for the presence of functional carbonic anhydrase. The patients of Soriano et al. (18) doubled their bicarbonate excretion after a single dose of acetazolamide in contrast to the patient from Holland (?) where no definite response was found even with a dose of 25 mg/kg. This latter finding was taken to indicate the absence of normal carbonic anhydrase. Our patient exhibited no increase in bicarbonate excretion at an ordinary dose of acetazolamide. On the contrary, a somewhat lowered excretion was found (Table 2). When, however, the acetazolamide dose was doubled (25 mg/kg), a significant decrease of $Tm–HCO_3$ and thus an increase of bicarbonate excretion occurred at both serum bicarbonate levels around 10 (data not presented) and above 20 mmol/l (Table 2). Responsive carbonic anhydrase seems thus to be present in B. H. A quantitative or qualitative defect in its activity might nevertheless be present, especially as the response to an ordinary acetazolamide dose was abnormal. Enzymatic and electron microscopic studies

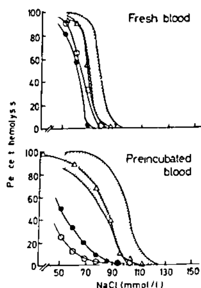


Fig. 3. Osmotic fragility of erythrocytes. Heparinized blood was incubated for 2 hours at room temperature with various dilutions of buffered saline. The test was performed within 30 min. after blood collection and after 24 hours incubation of the blood at 37 C. Circles represent the patients B. H. and O. H. and triangles their normal brother S. H.

Table 2 *The effect of acetazolamide on bicarbonate reabsorption at near normal serum bicarbonate level*

Sodium bicarbonate was infused at a constant rate of 0 83 mmole per min during both studies I and II Acetazolamide (Diamox®) was given intravenously at the start of collection period 4

Study no	30-min period	Acetazolamide (mg/kg b w)	Serum (mmol/l) HCO$_3$	Serum (mmol/l) K	Diuresis (ml/min)	GFR (ml/min)	Bicarbonate (mmol/100 ml GF) reabsorbed	Bicarbonate (mmol/100 ml GF) excreted
I	1	–	2	2 4	[illegible] 5	[illegible]6	1 [illegible]2	0 98
		–	23	2 4	3 2	[illegible]8	1 [illegible]6	1 04
	3	–	24	[illegible] 4	3 3	[illegible]6	1 27	1 13
	4	1[illegible]	6	[illegible] 1	[illegible] 8	20	1 69	0 91
	5	1	27	2 1	2 7	2	2 23	0 47
II	1	–	[illegible]1	[illegible] 4	4 2	28	1 15	0 95
		–	[illegible]1	–	5 0	38	1 13	0 97
	3	–	2[illegible]	[illegible] 3	4 7	33	1 13	1 07
	4	[illegible]5	[illegible]3	[illegible] 3	6 [illegible]	29	0 5[illegible]	1 78
	5	[illegible]5	[illegible]5	–	7 9	40	0 97	1 53
	6	[illegible]5	5	[illegible] 3	7 8	34	0 77	1 73

could clarify this question but we have not found it justified to take a kidney biopsy

One of the unique ways in which the kidney can provide H$^+$ for acid excretion or reabsorption of HCO$_3^-$ is by decarboxylation of alpha ketoglutarate producing CO$_2$ One may speculate whether the increased excretion of alpha ketoglutarate found in our patients is due to lowered decarboxylation of this compound in this type of RTA where less than half the normal amount of HCO$_3^-$ is presented to the tubules for reabsorption In favour of this is the finding of reduced alpha ketoglutarate excretion during treatment of B H with alkali (see below)

Parathormone is known to reduce proximal bicarbonate reabsorption by a mechanism other than inhibition of carbonic anhydrase possibly indirectly by a reduction of the proximal sodium and fluid absorption (14) The important role of parathormone in regulation of HCO$_3$ reabsorption is shown by the occurrence of systemic acidosis due to bicarbonate wastage in patients with hyperparathyreoidism (9 16) Our patient B H had normal serum levels of parathormone and no clinical signs of hyperparathyreoidism Administration of parathormone (6 I U /kg) resulted in a 3 fold increased diuresis 2 fold increased GFR and 7 fold increased natriuresis but no bicarbonaturia which agrees well with an inhibited reabsorption of sodium being the primary event (14)

Both the endogenous creatinine and inulin clearances were moderately decreased as was the effective renal plasma flow as estimated by ^{131}I Hippuran renography (Table 1) The urinary concentrating ability was also slightly reduced (Table 1) The reason for these reductions is not clear Intravenous pyelography was normal Microscopic examination of the kidneys of O H did not reveal glomerular or collecting duct pathology nor any sign of interstitial calcinosis as frequently found in distal but not usually in proximal RTA (10 20) Contraction of the extracellular fluid volume which might have been beneficial because of its effect on bicarbonate reabsorption (12) is probably not causative as normalization of GFR was not observed during bicarbonate infusion (Table 2) Theoretically slight hypokalaemia could explain the reduced concentrating ability However improvement was not observed during treatment with K$_3$ citrate Habitually increased drinking was not causative as diuresis was normal (Table 1)

Attempts to correct the acidosis of B H by peroral medication with K$_3$-citrate (14 mmol×

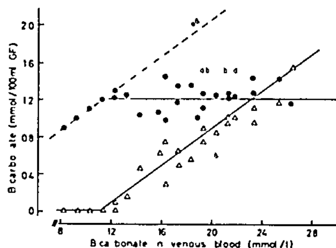


Fig 2 Relation between venous blood concentration renal tubular reabsorption and urinary excretion of bicarbonate in B H The stippled line represents the theoretical line of 100% reabsorption Filled symbols represent reabsorbed and open symbols excreted bicarbonate

amount of fluid given during the bicarbonate infusion studies in B H was similar to the volume of urine voided (1 500–1 800 ml) Furthermore the serum creatinine concentration remained remarkably constant 53–49–51 mmol/l during the bicarbonate infusion Serum Na increased from 136 to 145 mmol/l

The bicarbonate infusion normalized both serum bicarbonate and chloride whereas serum potassium decreased rapidly from 3 5 to a dangerously low level at 2 mmol/l This precipitous serum K fall is probably due to an increased Na–K exchange within the distal tubules presented with great amounts of $NaHCO_3$ Bicarbonate administration is known to increase the serum level of parathormone leading to phosphaturia (5) possibly explaining the development of pronounced hypophosphataemia with a fall from 2 1 to 0 23 mmol/l

In healthy individuals with normal bicarbonate levels the proximal tubules reclaim 85–90% of the filtered bicarbonate The enzyme carbonic anhydrase present both in the brush border and the interior of the proximal tubular cells is thought to be responsible for most of this reabsorption (7 17) Thus when the carbonic anhydrase inhibitor acetazolamide is given an increased bicarbonate excretion in urine normally occurs Such a response represents indirect evidence for the presence of functional carbonic anhydrase The patients of Soriano et al (18) doubled their bicarbonate excretion after a single dose of acetazolamide in contrast to the patient from Holland (?) where no definite response was found even with a dose of 25 mg/kg This latter finding was taken to indicate the absence of normal carbonic anhydrase Our patient exhibited no increase in bicarbonate excretion at an ordinary dose of acetazolamide On the contrary a somewhat lowered excretion was found (Table 2) When however the acetazolamide dose was doubled (25 mg/kg) a significant decrease of $Tm–HCO_3$ and thus an increase of bicarbonate excretion occurred at both serum bicarbonate levels around 10 (data not presented) and above 20 mmol/l (Table ?) Responsive carbonic anhydrase seems thus to be present in B H A quantitative or qualitative defect in its activity might nevertheless be present especially as the response to an ordinary acetazolamide dose was abnormal Enzymatic and electron microscopic studies

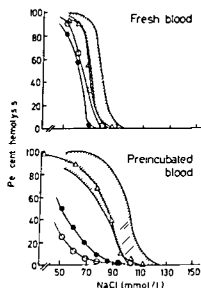


Fig 3 Osmotic fragility of erythrocytes Heparinized blood was incubated for 2 hours at room temperature with various dilutions of buffered saline The test was performed within 30 min after blood collection and after 24 hours incubation of the blood at 37°C Circles represent the patients B H and O H and triangles their normal brother S H

?1 days against 24 days as the lower normal limit This is similar to the findings of Pavone et al (13) in a patient with Lowe s syndrome They suggested an extra erythrocytic factor as being responsible However the finding of abnormally increased osmotic resistance of the erythrocytes (Fig 3) would in our patients probably favour a change in some intrinsic property of the erythrocyte membrane itself This is because incubation of normal erythrocytes in the patients serum did not result in increased osmotic resistance neither did incubation of the patients erythrocytes with normal serum lower the osmotic resistance In this connection it is of interest that Morris et al (8) because of the association of familial RTA and elliptocytosis suggest the possibility that the cell membrane of renal tubular cells and erythrocytes share a common protein

Increased osmotic resistance has been described both in iron deficiency anaemia thalassaemia and cholestasis (3) In these conditions the erythrocytes have a greater than normal membrane surface area in relation to the intracellular volume allowing a greater than normal volume expansion before bursting MCHC was just below the lower normal limit in our patients possibly explaining some of the increased osmotic resistance There were no signs of cholestasis and electrophoresis of haemoglobin from B H was normal We also found no increase of the erythrocytic cholesterol phospholipid ratio as in target cells (3)

CONCLUSION

It is clear that the degree of acidosis in primary proximal RTA may vary greatly from one patient to another In our siblings both the acidosis and the clinical consequences are severe

Except for growth retardation the clinical and biochemical characteristics of our patients are different from those of other published patients with either proximal or distal RTA although several features are similar to those of Lowe s syndrome (12 13 20) In the oculocerebrorenal syndrome of Lowe however multiple tubular defects (Fanconi syndrome) are present and the acidosis is not as severe as in our patients

The clinical condition in our patients is suggestive of a generalized enzyme or membrane defect i e present both in the central nervous system cornea lens erythrocytes and kidney tubules Another possibility is that several if not all of the features are secondary to the severe chronic HCl acidosis

REFERENCES

1 Brenes L G Brenes J N & Hernandez M M Familial proximal renal tubular acidosis A distinct clinical entity *Am J Med 63* 244 1977

? Donckerwolcke R A van Stekelenburg G J & Tiddens H A A case of bicarbonate losing renal tubular acidosis with defective carboanhydrase activity *Arch Dis Child 45* 769 1970

3 Cooper R A & Jandl J H Bile salts and cholesterol in the pathogenesis of target cells in obstructive jaundice *J Clin Invest 47* 809 1968

4 Graziani G De Vecchi A & Rosti D Primary proximal renal tubular acidosis A therapeutical approach and long term follow up *Hel Paediatr Acta 31* 4 7 1976

5 Mercado A Slatopolsky E & Klahr S On the mechanisms responsible for the phosphaturia of bicarbonate administration *J Clin Invest 56* 1386 1975

6 McSherry E & Morris R C Jr Attainment and maintenance of normal stature with alkali therapy in infants and children with classic renal tubular acidosis *J Clin Invest 61* 509 1978

7 Morris R C Jr Renal tubular acidosis Mechanisms classification and implications *N Engl J Med ?81* 1405 1969

8 Morris R C Jr Sebastian A & McSherry E Renal acidosis *Kidney Int 1* 3 ? 1972

9 Muldowney F P Carroll D V Donhoe J F & Freanay R Correction of renal bicarbonate wastage by parathyroidectomy *Q J Med XL* 478 1971

10 Narins R C & Goldberg M Renal tubular acidosis Pathophysiology diagnosis and treatment *DM 23* 1977

11 Nash M A Torrado A D Greifer I Spitzer A & Edelmann C M Jr Renal tubular acidosis in infants and children *J Pediatr 80* 738 1977

1 Oetliker O & Rossi E The influence of extracellular fluid volume on the renal bicarbonate threshold A study of two children with Lowe s syndrome *Pediatr Res 3* 140 1969

13 Pavone L Musumeci S Li Volti S Distefano G & Mollica F Haematological studies in a case of oculocerebrorenal syndrome *Helv Paediatr Acta 31* 509 1976

3) and Na bicarbonate (18 mmol×3) resulted in mean increments of pH from 7.12 to 7.24 and standard bicarbonate from 10 to 13 mmol/l, but the growth rate did not improve. A complete correction of the acidosis in our patient B. H. would be impossible by peroral treatment with $NaHCO_3$, as it probably would require more than 1000 mmoles daily as estimated from the bicarbonate infusion studies.

The beneficial effect of diuretics in proximal RTA is probably due to contraction of the extracellular volume which increases the tubular reabsorptive capacity for bicarbonate (4, 12, 15). However, therapeutic trials in our patient have been unsuccessful.

Clinical characteristics

Bicarbonate wasting of the degree present in this case is rare. To our knowledge, the only case similar to our patients is that of Donckerwolcke et al. (2). Their patient, a girl, had some of the features of our patients, i.e. mental and growth retardation. She had also corneal changes, but no nystagmus.

Growth failure is regularly present in RTA (6, 10, 20). The growth rate improves markedly when the acidosis is corrected, indicating that the acidosis itself may be causative (6). Intrauterine growth was normal in our patients, whereas growth was retarded in the first couple of years. From about 1–2 years the growth rate has been near normal in B. H., possibly due to adaptation to the chronic acidosis.

Mental retardation has not been regularly found in cases of RTA except in the oculo-cerebro-renal syndrome of Lowe (10, 12, 20) where it is more severe than in our patients. In various types of chronic metabolic acidosis due to inborn errors of metabolism resulting in accumulation of organic acids, mental retardation is common and probably due to the metabolic block with either accumulation or deficiency of metabolites. In the "pure" HCl acidosis of our patients no specific metabolic block outside the kidney is obvious, although several organs are affected. It seems conceiveable that the extremely low pH, probably present since birth, might adversely interfere at the biochemical level resulting in disturbed psychomotoric development and function.

The congenital nystagmus present in our patients has not been present in other cases of severe chronic acidosis. The opacities in cornea have been present since the age of 3½ and 5 years in the two brothers, and seem to increase with age. Corneal changes, although somewhat different (described as band keratopathy) were also present in the patient of Donckerwolcke et al. (2). The vision has deteriorated during the last years in B. H. due to progression of the cataract. At the age of eleven, bilateral glaucoma was diagnosed in B. H. Glaucoma has not been reported to develop in RTA, except for patients with Lowe's syndrome, where it usually is congenital (17, 13, 20).

Rather severe enamel defects were present in the permanent teeth of B. H. According to the dentists it is obvious that these defects originate from the period of enamel development and are not due to exogenous causes after eruption. This is in agreement with the fact that the primary dentition was normal in both patients. Our patients have no rickets, and except for moderately retarded bone age, skeletal X-ray examinations have been normal.

Biochemical and haematological data

Both our patients had pathologically low PBI and thyroxine values, and probably related to this, the thyroid gland was very small in O. H. In the girl described by Donckerwolcke et al. (2) PBI was also in the low normal range. Due to low stature and low PBI values O. H. was treated with l-thyroxine 0.05 mg daily for more than one year without any effect on the growth rate. The patients did not exhibit any clinical signs of hypothyroidism except for reduced height.

The haematological status was usually normal in both boys except for a slight reticulocytosis. $^{51}CrO_4^-$ labelling of the erythrocytes of B. H. revealed a mean survival of 20–

?1 days against 24 days as the lower normal limit This is similar to the findings of Pavone et al (13) in a patient with Lowe s syndrome They suggested an extra erythrocytic factor as being responsible However the finding of abnormally increased osmotic resistance of the erythrocytes (Fig 3) would in our patients probably favour a change in some intrinsic property of the erythrocyte membrane itself This is because incubation of normal erythrocytes in the patients serum did not result in increased osmotic resistance neither did incubation of the patients erythrocytes with normal serum lower the osmotic resistance In this connection it is of interest that Morris et al (8) because of the association of familial RTA and elliptocytosis suggest the possibility that the cell membrane of renal tubular cells and erythrocytes share a common protein

Increased osmotic resistance has been described both in iron deficiency anaemia thalassaemia and cholestasis (3) In these conditions the erythrocytes have a greater than normal membrane surface area in relation to the intracellular volume allowing a greater than normal volume expansion before bursting MCHC was just below the lower normal limit in our patients possibly explaining some of the increased osmotic resistance There were no signs of cholestasis and electrophoresis of haemoglobin from B H was normal We also found no increase of the erythrocytic cholesterol phospholipid ratio as in target cells (3)

CONCLUSION

It is clear that the degree of acidosis in primary proximal RTA may vary greatly from one patient to another In our siblings both the acidosis and the clinical consequences are severe

Except for growth retardation the clinical and biochemical characteristics of our patients are different from those of other published patients with either proximal or distal RTA although several features are similar to those of Lowe s syndrome (12 13 20) In the oculocerebrorenal syndrome of Lowe however multiple tubular defects (Fanconi syndrome) are present and the acidosis is not as severe as in our patients

The clinical condition in our patients is suggestive of a generalized enzyme or membrane defect i e present both in the central nervous system cornea lens erythrocytes and kidney tubules Another possibility is that several if not all of the features are secondary to the severe chronic HCl acidosis

REFERENCES

1 Brenes L G Brenes J N & Hernandez M M Familial proximal renal tubular acidosis A distinct clinical entity *Am J Med 6?* 244 1977
2 Donckerwolcke R A van Stekelenburg G J & Tiddens H A A case of bicarbonate losing renal tubular acidosis with defective carboanhydrase activity *Arch Dis Child 45* 769 1970
3 Cooper R A & Jandl J H Bile salts and cholesterol in the pathogenesis of target cells in obstructive jaundice *J Clin Invest 47* 809 1968
4 Graziani G De Vecchi A & Rosti D Primary proximal renal tubular acidosis A therapeutical approach and long term follow up *Hel Paediatr Acta 31* 4?7 1976
5 Mercado A Slatopolsky E & Klahr S On the mechanisms responsible for the phosphaturia of bicarbonate administration *J Clin Invest 56* 1386 1975
6 McSherry E & Morris R C Jr Attainment and maintenance of normal stature with alkali therapy in infants and children with classic renal tubular acidosis *J Clin Invest 61* 509 1978
7 Morris R C Jr Renal tubular acidosis Mechanisms classification and implications *N Engl J Med 281* 1405 1969
8 Morris R C Jr Sebastian A & McSherry E Renal acidosis *Kidney Int 1* 3 . 197?
9 Muldowney F P Carroll D V Donhoe J F & Freanay R Correction of renal bicarbonate wastage by parathyroidectomy *Q J Med XL* 478 1971
10 Narins R G & Goldberg M Renal tubular acidosis Pathophysiology diagnosis and treatment *DM 23* 1977
11 Nash M A Torrado A D Greifer I Spitzer A & Edelmann C M Jr Renal tubular acidosis in infants and children *J Pediatr 80* 738 197?
12 Oetliker O & Rossi E The influence of extracellular fluid volume on the renal bicarbonate threshold A study of two children with Lowe s syndrome *Pediatr Res 3* 140 1969
13 Pavone L Musumeci S Li Volti S Distefano G & Mollica F Haematological studies in a case of oculocerebrorenal syndrome *Helv Paediatr Acta 31* 509 1976

14 Puschett J B & Zurbach P Acute effects of parathyroid hormone on proximal bicarbonate transport in the dog *Kidney Int* 9 501 1976
15 Rampini S Fanconi A Illig R & Prader A Effect of hydrochlorothiazide on proximal renal tubular acidosis in a patient with idiopathic de Toni Debré Fanconi syndrome *Helv Paediatr Acta* 23 13 1968
16 Siddiqui A A & Wilson D R Primary hyperparathyroidism and proximal renal tubular acidosis Report of two cases *Can Med Assoc J* 106 654 1972
17 Soriano J R The renal regulation of acid base balance and the disturbances noted in renal tubular acidosis *Pediatr Clin North Am* 18 529 1971
18 Soriano J R Boichis H Stark H & Edelmann C M Jr Proximal renal tubular acidosis A defect in bicarbonate reabsorption with normal urinary acidification *Pediatr Res* 1 81 1967
19 Soriano J R Boichis H & Edelmann C M Jr Bicarbonate reabsorption and hydrogen ion excretion in children with renal tubular acidosis *J Pediatr* 71 802 1967
20 Soriano J R & Edelmann C M Jr Renal tubular acidosis *Annu Rev Med* 20 363 1969

Submitted Sept 11 1978
Accepted April 25 1979

(A W) Barneklinikken
Rikshospitalet
Oslo 1
Norway

Acta Pædiatr Scand 68 869-873 1979

227 ROAD ACCIDENTS TO CHILDREN

CYNTHIA M ILLINGWORTH

*From the Paediatric Accident and Emergency Department
Children s Hospital Sheffield England*

ABSTRACT Illingworth C M (Accident and Emergency Department Children s Hospital Sheffield England) 227 Road accidents to children Acta Paediatr Scand 68 869 1979 —In a 9 month period 227 children attended the Accident and Emergency Dept of the Children s Hospital Sheffield after accidents on the road—about 12% of all new attendances 169 were accidents to pedestrians 31 to cyclists and 27 to passengers Of the 169 pedestrian accidents 157 were hit by moving vehicles 72 (45 9%) suffering serious injury with two dead 45% of the pedestrians 22 6% of the cyclists and 11 1% of the passengers were admitted 29 6% of the 227 had severe head injury (concussion with or without a fractured skull) 22% of the 227 had a fracture or fractures 55 children had had previous accidents Compared with 225 previously described skateboard injuries and 200 playground equipment injuries those in jured on the roads were much more serious though with fewer fractures 37% of the road accidents were serious compared with 10 7% of those injured on skateboards and 7 5% of the play equipment cases 40 9% of the skateboard injuries but only 22 0% of the road accidents involved fractures but 29 6% of the latter 6 0% of the play equipment injuries and 0 9% of the skateboard injuries involved serious head injury 4 0% of the skateboard injuries 10 0% of the play equipment injuries but 45 0% of the pedestrian road accident cases necessitated admission

KEY WORDS Road accidents children

Accidents are the main cause of death of children aged 1–15 and the cause of a vast amount of morbidity often with much pain and psychological distress and they cause great anxiety to their parents Road accidents are an important cause of morbidity and mortality in children and in the Accident and Emergency Department of the Children s Hospital Sheffield which deals with medical and surgical emergencies road accidents in the period of this study contributed approximately 12% of all new attendances (which total approximately 25 000 per year)

PATIENTS AND METHODS

With the help of a proforma details were recorded of 227 consecutive new attendances at the Accident and Emergency Department of the Children s Hospital on account of accidents on the road paying particular attention to the way in which the accident happened with details from witnesses and the child and to the nature of the injuries The accidents covered a period of 9 months The children were seen by the Consultant in charge (C M I) or other Accident and Emergency staff which consisted of two part time Registrars and four Senior House Officers The mean age of the children was 7 9 years just over half being age 5 to 9 and a third 10 to 15 169 children were on foot 27 were passengers and 31 were on bicycles 62 of the pedestrians and 93% of the cyclists were boys The severity of the injuries was graded as follows

Grade 1 trivial usually no treatment or follow up necessary

Grade 2 minor lacerations soft tissue injuries or bruises

Grade 3 more serious lacerations undisplaced limb fractures or minimally displaced greenstick fractures and minor head injuries

Grade 4 fractures for which admission was essential seriously displaced fractures that needed manipulation under a general anaesthetic and head injuries with concussion or skull fracture or both

Grade 5 potentially life threatening conditions—for example ruptured viscus or serious head injuries The grading was done by the Consultant (C M I) and was made when the child s treatment was completed or all the facts about the injuries were known The grading was the same as that previously used in a paper on playground equipment injuries (1) and skateboard injuries (2) so that comparisons between the results of accidents could be made

Table 1. *Comparison of injuries on skateboards, playground equipment, roads*

	Source of Injury					
			Road			
	Skate board	Playground equipment	Pedestrian	Passenger	Cycle	Total
Mean age (years)	11.1	6.3	7.6	8.0	9.4	7.9
Number of cases	225	200	169	27	31	227
Injury grade 1 %	1.8	28.5	0	7.4	0	0.9
Injury grade 2 %	44.0	45.0	21.3	14.8	29.0	21.6
Injury grade 3 %	43.9	19.0	34.9	66.7	48.4	40.5
Injury grade 4 %	10.7	6.5	33.7	3.7	16.1	27.8
Injury grade 5 %	0	1.0	8.9	3.7	6.5	7.9
Died %	0	0	1.2	3.7	0	1.3
Fractures %	40.9	26.5	26.0	7.4	16.1	22.0
Concussion with or without skull fracture %	0.9	6.0	33.1	11.1	27.6	9.6
Soft tissue injuries alone %	33.8	22.5	23.7	19.4	22.2	22.9
Admitted %	4.0	10.0	45.0	11.1	22.6	37.9
Injuries above neck %	15.6	46.0	67.4	70.4	77.4	69.[illegible]
X-rays taken %	68.4	59.5	68.6	33.3	67.8	67.3

Head injuries were sub-divided into three groups—severe, denoting concussion or fractured skull or both; minor, denoting significant haematoma or laceration over the skull; and other injuries above the neck—abrasions and less severe abrasions, bruises, nose bleeds etc.

Of the 227 children, 20 came from outside the city boundary. Approximately one third were going to or from school and just over a half were on holiday, in the week-ends or outside school hours, but figures for this are not accurate. At least 6 were known to come from problem families.

RESULTS

Table 1 shows the grading of the injuries: 0.9% in grade 1, 21.6% in grade 2, 40.5% in grade 3, 27.8% in grade 4 and 7.9% in grade 5 with 3 deaths (1.3% of the total). It will be seen that 43.8% of the pedestrians, 22.6% of the cyclists and 11.1% of the passengers had the most serious accidents—grades 4, 5 or

Table 2. *Road accidents: details*

	Pedestrian (*n*=169) (%)	Passenger (*n*=27) (%)	Cycle (*n*=31) (%)	Total 227 (%)
Head injury: Severe				
Solely	7.1	7.4	6.5	7.5
Fractured skull	3.6	3.7	6.5	4.0
Other fracture	9.5	0	0	7.1
Other soft tissue injury	13.0	0	9.7	11.0
Head injury: Minor				
Solely	10.1	30.0	9.7	12.3
Fracture elsewhere	2.4	0	6.5	2.7
Other soft tissue injury	14.8	3.7	19.4	14.1
Other injury above neck: bruises, lacerations, abrasions				
Solely	0.6	22.2	9.7	4.4
Fracture elsewhere	1.2	0	0	0.1
Other soft tissue injury	5.3	3.7	9.7	5.7
Fractures other than above	8.9	7.4	3.2	7.9
Soft tissue injury other than above	23.7	22.2	19.4	22.9

death But of the 169 pedestrians 12 had merely fallen on the road 9 of them on ice and had not been hit by a vehicle only two of those had worse than a grade 2 or 3 injury Of the 157 hit by a moving vehicle 72 (45 9%) had a grade 4 or 5 injury and two died 37 9% of the 227 were admitted 45 0% of the pedestrians 22 6% of the cyclists and 11 1% of the passengers

Approximately two-thirds of the pedestrians and three quarters of the cyclists and passengers had injuries above the neck 29 6% of the 227 had a severe head injury the figure for pedestrians was 33 2% for passengers 11 1% and for cyclists 22 7% 4% of the total had a fractured skull and 7 1% of those with a severe head injury had fractures elsewhere Those with severe head injury were admitted but 27 of the 66 with minor head injuries were allowed home after a period of observation in the Department and with the usual head injury instructions Table 2 shows that of the 227 children 22 0% had fractures (skull or elsewhere) and 22 9% had soft tissue injuries other than those above the neck Several had multiple fractures Four had injury to the kidney with haematuria and 2 had rupture of the spleen 7 others had abdominal injury and one had laceration of the anus and rectal mucosa

X rays were taken in approximately two thirds of the children but in 39% of the head injuries an X ray of the skull was not thought necessary

Fifty five of the children had had previous accidents (24 2%) their mean age was 8 2 years two-thirds were boys Twenty of the 55 had had two or more accidents and 8 had had four or more Five came from known problem families

Under *Causes of Accidents* I have described the ways in which the accidents happened In many cases owing to retrograde amnesia it was impossible to obtain an account from the child but inaccurate as it must be we tried our best to determine how the accidents happened Several children admitted that they had not looked before crossing the road or that they looked one way only In at least 22 cases the child s vision was obscured by stationary vehicles—in 19 cases the child passed between buses and cars and was then hit by a vehicle Eighteen said that they had not seen an approaching vehicle in two cases it was foggy but in three cases the car came round a corner and could not be seen approaching and in one case a car performed an unexpected U turn Eight children were hit when running across the road to friends at the other side At least 12 were playing on the road hiding chasing or being chased Six were hit on a pedestrian crossing Two were deaf It is impossible to say how many accidents were due to the child with his inexperience under estimating the speed of a car One boy said he was crossing t road when this raving idiot came and knocked me half a mile up t road!

As for passengers 3 fell out of a moving car on a corner and 2 were injured in a stationary car when the car was hit in the rear by another vehicle Three of the accidents involved babies in prams In one case a pram pushed by a 15 year-old aunt was hit by a car In another a father pushing the pram misjudged traffic lights the pram was hit and the baby killed

The cycle accidents were due to the usual causes—collisions at a road junction hitting an obstruction or skidding In one case a wheel came off in another the boy braked too violently and went over the handlebars

Causes of Accidents

Pedestrian (total 169)

Falls on road only not hit by vehicle 12 (9 fell on ice one fell down a bank onto moving car) all others (157) hit by vehicle
Hit by vehicle no other information 51
Admitted not looking 13
Admitted not seeing car 18 (? in fog car came round corner 3 car performing U turn 1)
Friends or mother on opposite side child running to them 8
Admitted running across road 13 (? hit by police car one fell off parked lorry ran across road and hit one fell when running across and was run over)
Playing on road hiding behind car chasing or being chased 12

Vision obscured 22 (19 crossing from between buses or cars, 3 from behind ice cream van)
Pedestrian or supervised crossing 6 (one car or bus stopping, another vehicle failing to stop; one child was crossing with mother, both hit)
Pedestrian crossing had been moved, child crossed, hit
Deaf 2
Ran behind backing car 2, in front of parked car when it moved 2
Jumped off wall onto moving car 1
Ran down bank, could not stop, hit by car
Hesitation: children ran into road, car stopped, car restarted, children ran back, hit 2

Cyclist

Hit by car (car came from a turning 1, cyclist failed to give way at junction 2, cyclist crossed road without looking 1)
Cycling across pedestrian crossing, hit
Hit by skidding car 1, by reversing car 1
Collided with another cycle 1
Wheel came off 1
Braked too violently, went over handlebars 1
Hit stone, wall, curb 6
Just fell off 3
Skidded 2

Passenger

Car collision 16
Fell out of moving car 3
Fell off milk trolley 1, off motorcycle pillion 1, off bus 1
Sitting in stationary car, car hit in rear 2

Comparison with Two Previous Studies

Table 1 compares the present data with those of two previous studies, one of 225 skateboard injuries and the other of 200 playground equipment injuries (1, 2). The mean age of the skateboarders was higher (11.1 years) than that in the other two studies. It will be seen that 45.8% of the skateboard injuries and 73.5% of the play injuries fell into grades 1 and 2 compared with 22.5% of the road accidents, and only 10.7% of the skateboard accidents and 7.5% of the play injuries fell into grades 4 and 5, the most serious, compared with 37.0% of the road accidents (three of which were fatal). The percentage of fractures (40.9%) was almost double that in the road accident cases, but skateboard accidents led to serious head injuries in 0.9%, play equipment in 6.0%, and road accidents in 29.6%. Only 4.0% of the skateboard accidents led to admission, 10.0% of the play injuries, but 37.9% of the road accidents (including 45.0% of the pedestrian injuries).

DISCUSSION

The serious problem of accidents to children on the roads is indicated by the fact that 66 of the 227 children suffered concussion with or without a fractured skull, that 63 children (57 of them pedestrians) had grade 4 accidents, 18 (grade 5) suffered life threatening injury, and three died. More than two thirds of the children had injuries above the neck.

These are not new problems but the publicity given to them is minute compared with that given to accidents on skateboards and playground equipment—which have less than half as high a percentage of most serious injuries. Accidents on skateboards resulted in a much higher number of fractures (40.9%) than accidents on the roads (22.0%) but the majority of the skateboard fractures were comparatively trivial compared with the fractures resulting from road accidents.

It is easy to blame children for inviting injury by playing on the road—but they may have no other place. They may misjudge the speed of traffic and many of them are not old or mature enough to be able to do so accurately. Many adults are not good at this either! The children's small size makes it impossible for them to see over parked cars so that they often emerge as from a tunnel into fast moving traffic. Young children are easily distracted, especially when they are with other children, so that if they see someone or something interesting on the other side of the road they may rush across and forget completely that there may be dangers in the way. Running home after school they may be tired and almost certainly hungry; both will reduce their powers of concentration and alertness.

Parents may be to blame when they leave young children in potentially dangerous situations which are too complex for them to deal with at their stage of development, but chil

dren have to be allowed to grow up and acquire independence Over protection has the opposite of the effect desired leading to accident proneness It is easy to blame parents but even the most careful parents have to some degree to take calculated risks with their children Children must have some experience of traffic and exposure to traffic in order to become competent to deal with it Many children have the sort of personality and home background which makes them more likely to have accidents and some children have physical handicaps such as deafness which increase the risk

As for drivers we cannot say how many of the accidents were due to alcohol or drugs we lag behind other countries in the prevention of driving under the influence of alcohol

ACKNOWLEDGEMENTS

I would like to thank the junior medical staff of the Paediatric Accident & Emergency Department at the Children s Hospital Sheffield for filling in the proformas

REFERENCES

1 Illingworth C Brennan P Jay A Al Rawi F & Collick M 200 injuries caused by playground equipment *Br Med J II* 33? 1975

? Illingworth C Jay A Noble D & Collick M 2_5 Skateboard injuries in children *Clin Pediatr 17* 781 1978

Submitted Oct 16 1978
Accepted Febr ?3 1979

Paediatric Accident and Emergency Department
Children s Hospital
Sheffield 10
England

Vision obscured 22 (19 crossing from between buses or cars, 3 from behind ice cream van)
Pedestrian or supervised crossing 6 (one car or bus stopping, another vehicle failing to stop; one child was crossing with mother, both hit)
Pedestrian crossing had been moved, child crossed, hit
Deaf 2
Ran behind backing car 2, in front of parked car when it moved 2
Jumped off wall onto moving car 1
Ran down bank, could not stop, hit by car
Hesitation: children ran into road, car stopped, car restarted, children ran back, hit 2

Cyclist

Hit by car (car came from a turning 1, cyclist failed to give way at junction 2, cyclist crossed road without looking 1)
Cycling across pedestrian crossing, hit
Hit by skidding car 1, by reversing car 1
Collided with another cycle 1
Wheel came off 1
Braked too violently, went over handlebars 1
Hit stone wall, curb 6
Just fell off 3
Skidded 2

Passenger

Car collision 16
Fell out of moving car 3
Fell off milk trolley 1, off motor cycle pillion 1, off bus 1
Sitting in stationary car, car hit in rear 2

Comparison with Two Previous Studies

Table 1 compares the present data with those of two previous studies, one of 225 skateboard injuries and the other of 200 playground equipment injuries (1, 2). The mean age of the skateboarders was higher (11.1 years) than that in the other two studies. It will be seen that 45.8% of the skateboard injuries and 73.5% of the play injuries fell into grades 1 and 2 compared with 22.5% of the road accidents and only 10.7% of the skateboard accidents and 7.5% of the play injuries fell into grades 4 and 5, the most serious, compared with 37.0% of the road accidents (three of which were fatal). The percentage of fractures (40.9%) was almost double that in the road accident cases, but skateboard accidents led to serious head injuries in 0.9%, play equipment in 6.0%, and road accidents in 29.6%. Only 4.0% of the skateboard accidents led to admission, 10.0% of the play injuries, but 37.9% of the road accidents (including 45.0% of the pedestrian injuries).

DISCUSSION

The serious problem of accidents to children on the roads is indicated by the fact that 66 of the 227 children suffered concussion with or without a fractured skull, that 63 children (57 of them pedestrians) had grade 4 accidents, 18 (grade 5) suffered life threatening injury and three died. More than two thirds of the children had injuries above the neck.

These are not new problems but the publicity given to them is minute compared with that given to accidents on skateboards and playground equipment—which have less than half as high a percentage of most serious injuries. Accidents on skateboards resulted in a much higher number of fractures (40.9%) than accidents on the roads (22.0%) but the majority of the skateboard fractures were comparatively trivial compared with the fractures resulting from road accidents.

It is easy to blame children for inviting injury by playing on the road—but they may have no other place. They may misjudge the speed of traffic and many of them are not old or mature enough to be able to do so accurately. Many adults are not good at this either[1]. The children's small size makes it impossible for them to see over parked cars so that they often emerge as from a tunnel into fast moving traffic. Young children are easily distracted, especially when they are with other children, so that if they see someone or something interesting on the other side of the road, they may rush across and forget completely that there may be dangers in the way. Running home after school, they may be tired and almost certainly hungry; both will reduce their powers of concentration and alertness.

Parents may be to blame when they leave young children in potentially dangerous situations which are too complex for them to deal with at their stage of development, but chil

COMPUTED TOMOGRAPHIC FINDINGS OF THE BRAIN IN CHILDREN WITH ACUTE LYMPHOCYTIC LEUKEMIA AFTER CENTRAL NERVOUS SYSTEM PROPHYLAXIS WITHOUT CRANIAL IRRADIATION

S KOLMANNSKOG P J MOE and I M ANKE

From the Department of Paediatrics University of Tromsø Tromsø Department of Paediatrics University of Trondheim Trondheim and Department of Radiology University of Tromsø Tromsø Norway

ABSTRACT Kolmannskog S Moe P J and Anke I M (Department of Paediatrics University of Tromsø Tromsø Department of Paediatrics University of Trondheim Trondheim and Department of Radiology University of Tromsø Tromsø Norway) Computed tomographic findings of the brain in children with acute lymphocytic leukemia after central nervous system prophylaxis without cranial irradiation Acta Paediatr Scand 68 875 1979 —Nineteen children in primary remission of acute lymphocytic leukemia (ALL) were investigated by computed tomographic (CT) scans of the brain 2 to 64 (mean 19) months after the central nervous system (CNS) prophylaxis was finished The CNS prophylaxis consisted of high dose Methotrexate (HDM) intravenously combined with 6–8 doses of Methotrexate intrathecally Two children received only Methotrexate intrathecally as CNS prophylaxis In addition three children with ALL who had CNS leukemia were investigated by CT scans of the brain Only one abnormal CT scan was found among the nineteen asymptomatic children and one of the three patients with CNS relapse had slightly dilatated subarachnoidal spaces These results compared with other reports in literature in which the CNS prophylaxis has consisted of intrathecal Methotrexate and cranial irradiation suggest that there are fewer abnormal CT findings of the brain in patients not receiving cranial irradiation as part of CNS prophylaxis

KEY WORDS Acute lymphocytic leukemia CNS prophylaxis CT findings

entral nervous system (CNS) involvement as a usual event in the natural history of ute lymphocytic leukemia (ALL) (5 11) ne of the most important new steps in the eatment of children with ALL has therefore een the introduction of CNS prophylaxis (1) ntrathecal chemotherapy combined with ranial irradiation (usually 2400 rads) has been he most commonly used method However everal reports have shown the neurotoxicity f this combination (6 7 9 10 15 18–21) ven in asymptomatic children with ALL 50–0% have shown abnormal computed tomo raphic (CT) findings (2 17) Lowered intel ectual abilities among these patients has been lemonstrated (3)

CNS leukemia may also effectively be pre vented by using an adequate regimen of Methotrexate intrathecally (8 11) This meth od has been further improved by a combination of Methotrexate intrathecally and high dose Methotrexate (HDM) intravenously as part of a sanctuary phase This programme is now used on a national basis in Norway (12 13)

The purpose of this investigation has been to study the CT findings in children with ALL after CNS prophylaxis without cranial irradia tion particularly in cases receiving three courses of HDM CT scanners have just been obtained in three cities in Norway It has therefore not been possible to perform CT studies on all patients in Norway receiving HDM

PATIENTS AND METHODS

A total of nineteen children from 3 to 14 (mean 8) years in primary remission of ALL were investigated by CT scans of the brain 2 to 64 (mean 19) months after the CNS prophylaxis was finished Three children from 6 to 14 (mean 10) years with ALL and CNS leukemia (1 to 4

The question whether Methotrexate used alone will give adequate CNS prophylaxis has been discussed Moe et al have only observed 2 cases of CNS leukemia among 34 patients receiving Methotrexate intrathecally as the only type of CNS prophylaxis in the period 1971–1975 (11) Similar low incidence of CNS leukemia has been demonstrated by Hagbin et al (8) Only two of the first 69 children in Norway receiving HDM during their first re mission have so far developed CNS leukemia [13]

Because of the high prevalence of abnormal CT findings in children receiving both Metho rexate intrathecally and cranial irradiation as CNS prophylaxis it has been suggested that alternative ways of giving CNS prophylaxis should be considered (17) We have found few side effects from the CNS after CNS pro phylaxis consisting of Methotrexate intrathe cally and HDM and the results so far seem to be encouraging [13]

REFERENCES

1 Aur R J A Hustu H O Verzosa M S Wood A & Simone J V Comparison of two methods of preventing central nervous system leukemia *Blood* 4? 349 1973

Casteels Van Daele M Van de Casseye W Casaer P Eeckels R Palmers Y & Crolla D CT scans of the brain in children with acute lymphocytic leukemia after prophylactic treatment of the central nervous system In *Proceedings of the 10th Meeting of the International Society of Paediatric Oncology Brussel September 1978*

3 Eiser C Intellectual abilities among survivors of childhood leukemia as a function of CNS irradiation *Arch Dis Child 53* 391 1978

4 Enzmann D R & Lane B Cranial computed tomography findings in anorexia nervosa *J Comput Assist Tomog 1* 410 1977

5 Evans A E Gilbert E S & Zandstra R The in creasing incidence of central nervous system leuke mia in children *Cancer* 26 404 1970

6 Flament Durand J Ketelbant Balasse P Maurus R Regnier R & Spehl M Intracerebral calcifica tions appearing during the course of acute lympho cytic leukemia treated *with Methotrexate and X rays Cancer 35* 319 1975

7 Giralt M Gil J L Borderas F Oliveros A Gomez Pereda R Pardo J Martinez Ibanez F & Ra chs A Intracerebral calcifications in childhood lymphoblastic leukemia *Acta Haematol 59* 193 1978

8 Haghbin M Tan C T C Clarkson B D Miké V Burchenal J H & Murphy M L Treatment of acute lymphoblastic leukemia in children with pro phylactic intrathecal methotrexate and intensive systemic chemotherapy *Cancer Research 35* 807 1975

9 Kay H E M Knapton P J O Sullivan J P Wells D G Harris R F Innes E M Stuart J Schwartz F C M & Thompson E N Encephalo pathy in acute leukemia associated with methotrexate therapy *Arch Dis Child 47* 344 197?

10 McIntosh S Fisher D B Rothman S G Rosen field N Lobel J S & O Brien R T Intracranial calcifications in childhood leukemia *J Pediatr 91* 909 1977

11 Moe P J & Finne P H Intrakranielle leukemiske infiltrater hos barn *Tidsskr Nor Laegeforen 95* 874 1975

1? Moe P J & Seip M High dose methotrexate in acute lymphocytic leukemia in childhood *Acta Paediatr Scand 67* ?65 1978

13 Moe P J Høy dose metotrexat til barn med akutt lymfatisk leukemi *Tidsskr Nor Laegeforen 99* ?78 1979

14 Ochs J J Berger P E Brecher M L Sinks L F & Freeman A I Computed tomography (CT) scans in children with acute lymphocytic leukemia (ALL) following CNS prophylaxis without radio therapy *Proc ASCO 19* 391 1978

15 Oliff A Bleyer W A & Poplack D G Acute en cephalopathy after initiation of cranial irradiation for meningeal leukemia *Lancet I* 13 1978

16 Pedersen H Gyldensted M & Gyldensted C Mensuration of the normal ventricular system and supratentorial subarachnoidal space in children with computed tomography To be published

17 Peylan Ramu N Poplack D G Pizzo P A Ador nato B T & Di Chiro G Abnormal CT scans of the brain in asymptomatic children with acute lympho cytic leukemia after prophylactic treatment of the cen tral nervous system with radiation and intrathecal chemotherapy *N Engl J Med 298* 815 1978

18 Pochedly C Neurotoxicity due to CNS therapy for leukemia *Med Pediatr Oncol 3* 101 1977

19 Price R A & Jamieson P A The central nervous system in childhood leukemia *Cancer 35* 306 1975

?0 Rubinstein L J Herman M M Long T F & Wilbur J R Disseminated necrotizing leukoen cephalopathy A complication of treated central ner vous system leukemia and lymphoma *Cancer 35* ?91 1975

21 Young L W Intracerebral calcifications in treated leukemia in a child *Am J Dis Child 131* 1?84 1977

Submitted April 9 1979
Accepted June 5 1979

(S K) Department of Paediatrics
University of Tromsø
901? Tromsø
Norway

CNS relapses) were also investigated by CT scans of the brain 1 to 62 (mean 27) months after the CNS leukemia was diagnosed.

The induction phase treatment consisted of vincristine weekly and prednisone or dexamethasone daily followed by a ten days course of L-asparaginase. When remission had been achieved, three intravenous courses of HDM (500 mg/m²) were given at 3 weekly intervals. Maintenance therapy was then started with daily oral 6-mercaptopurine and weekly oral Methotrexate and pulses of steroid/vincristine (12).

CNS prophylaxis during the last 3½ years has consisted of three HDM courses intravenously and 6–8 doses of Methotrexate (12 mg/m²) intrathecally. Two patients received only Methotrexate intrathecally as CNS prophylaxis. None of the children has got cranial irradiation as part of the CNS prophylaxis.

The three children with CNS leukemia have not received CNS prophylaxis during the induction phase. After their CNS relapses were diagnosed, they have received Methotrexate intrathecally every month after new remission was achieved. They have been given from 5 to 60 doses of Methotrexate intrathecally at the time CT scans were performed.

The CT investigation of 16 children were performed with a Delta Scanner 50 FS, and six children were investigated with an EMI Scanner 5005.

Evaluation of the CT scans was done by a neuroradiologist (I. M. A.). The measurements of normal ventricular system and subarachnoidal spaces in children as described by Enzmann & Lane (4) and Pedersen et al. (16) were used.

RESULTS

Of the nineteen children in primary remission of ALL, an abnormal CT scan was only found in a single case, a four year old girl. Four days after the sixth intrathecal Methotrexate injection and five days after her second course of HDM intravenously, she had convulsions and lost consciousness. She developed a left sided hemiplegia and electroencephalographic abnormalities of her right hemisphere. The CT scan eight months later showed a small lesion with low and high density and without contrast enhancement in the region of the insula. One year later this abnormality could hardly be seen. She has later recovered almost completely from her hemiplegia and she has no signs of intellectual disturbances.

None of the three children with CNS relapses had enlarged ventricles. Even a girl with four CNS relapses and who had received 60 injections of Methotrexate intrathecally and two courses of HDM showed normal CT findings. Only one of them had slightly dilatated subarachnoidal spaces.

DISCUSSION

Different types of abnormalities have been described in patients who have received CNS prophylaxis consisting of cranial irradiation and intrathecal Methotrexate or cytosine arabinoside: ventricular dilatation, widening of the subarachnoidal spaces, areas of decreased attenuation coefficient and intracerebral calcifications (17). There was only demonstrated abnormal CT scan in one of the nineteen children in primary remission of ALL. Probably this abnormality was due to Methotrexate medication. Paresis following intrathecal instillation of Methotrexate has been described earlier (18).

Our number of CT studied cases who have received three courses of HDM are so far small. Ochs et al. (14) have, however, demonstrated pathological CT findings in only one of 35 cases receiving a similar type of combined CNS prophylaxis. These two independent studies strongly suggest that CT abnormalities are rare in children receiving this type of CNS prophylaxis without cranial irradiation. However, it remains to be shown that the CT abnormalities will be associated with signs of brain damage in children, although impairment of intellectual function has already been demonstrated after some years in a large proportion of children receiving irradiation to the CNS combined with Methotrexate intrathecally, while no abnormality was found in a group receiving only Methotrexate as prophylaxis (3).

Observation time is too short for the demonstration of possible late effects of HDM on intellectual functions. It is reassuring, however, that almost all such children seem to have normal CT scans when studied at least two months after CNS prophylaxis was finished. There is a need for larger series with CT scans and later follow up to substantiate this.

Acta Pædiatr Scand 68 879–885 1979

PHYSICAL HEALTH SCREENING OF SCHOOI CHILDREN

Extended Health Care Responsibilities for School nurses

R KORNFALT B JÖNSSON and I ROSLUND

F om th Dalb Communit Health Research Centre D lb Sweden and the Department of Paediatrics Universit Hospital Lund S eden

ABSTRACT Kornfalt R Jonsson B and Roslund I (Dalby Community Research Centre Dalby and Department of Paediatrics University Hospital Lund Sweden) Physical health screening of school-children Extended health care responsibilities for school nurses Acta Paediatr Scand 68 879 1979 —All 410 ten and twelve year old children of a school district underwent two repeated physical examinations within the school services the first by the school nurse the second by the school doctor The aim was to compare their assessments to see if physical class examinations could be delegated to the nurse in future in order to release doctor s time More than half of the children were found to have slight deviations from normal most common of the spine and in the skin The nurse detected many more deviations than the doctor but their assessments showed good agreement concerning functionally important deviations Newly detected functionally important deviations were noted in 8 children (2 %)

The routine physical examination could perfectly well be delegated to the school nurse who has the necessary prerequisites to take this responsibility and screen out those children in need of a doctor s assessment in this study 20% She would release valuable time for the doctor who could then apply himself to the real health problems of the children of today chronic diseases behavioural and school problems many of which frequently are concerns beyond the boundaries of traditional medical care

KEY WORDS School health physical health-screening school nurse nurse practitioners primary care

Changing health needs and changing concepts of health have made a re evaluation of School Health Programmes necessary Infectious diseases and nutritional defects are no longer major health problems Today accidents handicaps chronic diseases and emotional disturbances are more *important* The greatest problems of the School Health Service today are behavioural defects inadequate functioning in school and adjustment problems in adolescence (13)

The School Health Service which has the major responsibility for the health of school aged children has very often insufficient resources for today s greater need to work within all areas of the children s lives (2 3 6 14 20)

The Swedish School Health Service was organized in its present form (5) in 1944 The aim is to follow up the growth and development of school children to retain and improve their mental and physical health and to influence them to form healthy habits The most important aspect within the Service is preventive in character The programme includes periodic physical examinations vision and auditory screening and a vaccination programme The School Health staff consists of a physician visiting school once a week and a nurse usually fulltime The physician makes traditional physical examinations in the first fourth seventh and ninth classes of the elementary school i e at 7 10 14 and 16 years of age It has recently been suggested that the last two examinations should be exchanged for one only in the eighth class (24)

Acta Pædiatr Scand 68 879–885 1979

PHYSICAL HEALTH SCREENING OF SCHOOL CHILDREN

Extended Health Care Responsibilities for School nurses

R KORNFALT B JÖNSSON and I ROSLUND

From the Dalby Community Health Research Centre Dalby Sweden and the Department of Paediatrics University Hospital Lund Sweden

ABSTRACT Kornfält R Jonsson B and Roslund I (Dalby Community Research Centre Dalby and Department of Paediatrics University Hospital Lund Sweden) Physical health screening of school children Extended health care responsibilities for school nurses Acta Paediatr Scand 68 879 1979.—All 410 ten and twelve year old children of a school district underwent two repeated physical examinations within the school services the first by the school nurse the second by the school doctor The aim was to compare their assessments to see if physical class examinations could be delegated to the nurse in future in order to release doctor s time More than half of the children were found to have slight deviations from normal most common of the spine and in the skin The nurse detected many more deviations than the doctor but their assessments showed good agreement concerning functionally important deviations Newly detected functionally important deviations were noted in 8 children (2%)

The routine physical examination could perfectly well be delegated to the school nurse who has the necessary prerequisites to take this responsibility and screen out those children in need of a doctor s assessment in this study 20% She would release valuable time for the doctor who could then apply himself to the real health problems of the children of today chronic diseases behavioural and school problems many of which frequently are concerns beyond the boundaries of traditional medical care

KEY WORDS School health physical health screening school nurse nurse practitioners primary care

Changing health needs and changing concepts of health have made a re-evaluation of School Health Programmes necessary Infectious diseases and nutritional defects are no longer major health problems Today accidents handicaps chronic diseases and emotional disturbances are more important The greatest problems of the School Health Service today are behavioural defects inadequate functioning in school and adjustment problems in adolescence (13)

The School Health Service which has the major responsibility for the health of school aged children has very often insufficient resources for today s greater need to work within all areas of the children s lives (2 3 6 14 20)

The Swedish School Health Service was organized in its present form (5) in 1944 The aim is to follow up the growth and development of school children to retain and improve their mental and physical health and to influence them to form healthy habits The most important aspect within the Service is preventive in character The programme includes periodic physical examinations vision and auditory screening and a vaccination programme The School Health staff consists of a physician visiting school once a week and a nurse usually fulltime The physician makes traditional physical examinations in the first fourth seventh and ninth classes of the elementary school i e at 7 10 14 and 16 years of age It has recently been suggested that the last two examinations should be exchanged for one only in the eighth class (24)

Table 1 Classification of health problems

Health problem	Group 0 Healthy child	Group 1 Slight deviation without importance	Group 2 Moderate deviation treatment indicated	Group 3 Definitely handicapping disorders
Somatic development		Weight or height $> \pm 2$ S D	Obesity $> +3$ S D	Chronic disease e g severe asthma diabetes uncontrolled epilepsy etc
Skin abnormality		Warts naevi minor allergy extensive acne	Moderate allergic eczema Mycosis	Ichtyosis severe allergic eczema
Spine deformity		Bad posture static scoliosis kyfosis	Structural scoliosis $>10°$ deviation (Cobb)	Structural scoliosis with brace treatment or operation
Joint problems		Knock knees	Arthritis tendinitis Mb Schlatter	Rheumatoid arthritis
Feet problems		Pes planus		
Abnormality in motor development		Slight disturbances in fine and gross movements	Minor brain dysfunction (MBD) e g disturbances in fine and gross movements combined with perception difficulties	Cerebral palsy or other severe motor handicap

The value of having physicians performing the periodic routine physical examinations has been questioned several evaluations of the yield of these examinations have been discouraging (2 15 17 18 30 31)

The Swedish school physician however still spends more than half of his time on routine physical examinations (19) and has seldom time for the children s individual social or emotional problems The working conditions in the School Health Service are by many doctors conceived as unsatisfactory (27)

The school nurse is the main resource of the school health team She is in close contact with all the children in her area as well as with their teachers She comes to know the children well and learns of their problems when they come to her for minor ailments and accidents or to be weighed and measured vaccinated or have their vision tested The children find a refuge with her when they feel bad whatever the cause Her assessment of each child s health should therefore be at least as reliable as the doctor s Studies based on registrations of consultations with the Swedish school nurse show that she can handle 80% of the problems on her own without help from a doctor (16 19) It seems probable that she has the prerequisites to make correct assessments of the children s physical health also in routine examinations In this way valuable time would be released for the doctor who could then devote more interest to medical as well as psychosocial problems

In this study the periodic routine physical examinations were delegated to the school nurses Their assessments were compared with the doctor s who examined the same children with a traditional examination The authors have also studied the frequency of social and behavioural problems in the children based on parents children s and teachers interviews The results will be presented in a separate paper

MATERIALS AND METHODS

The material comprises all 10 year-old (n=223) and all 13 year old (n=187) children from the primary school of the Dalby area in southern Sweden

Details of the physical examinations of 10 year-old children have previously been reported (15) All the children were examined by the permanent school health team of the district 2 nurses and 1 physician The physician (R K) is a trained paediatrician with 3 years experience of school health work and a special interest in social paediatrics

The school nurses had 8 and 3 years of experience of school health work in the district respectively Prior to

Table 2 *Functionally important health problems in 410 10 to 12 year old children*

	10-year old n=223 (%)	12 year old n=187 (%)	Sum n=410 (%)
History and physical examination	11 7	15 1	13 4
Newly detected	0 9	2 1	1 5
Previously known	10 8	13 0	11 9
Vision examination	11 7	13 9	12 8
Newly detected	7	1 0	1 8
Previously known	9 0	12 9	10 9
Auditory examination	7	3 0	2 8
Newly detected	0 9	0 6	0 7
Previously known	1 8	2 4	2 1
Total	26 2	30 0	28 0
Newly detected	4 5	4 0	4 2
Previously known	21 7	26 0	23 8

the examination the nurses had repeated training sessions with demonstrations by the doctor A trained physiotherapist demonstrated how to make an examination of the spine In addition the nurses had access to a written manual of instructions

Each nurse examined about 00 children once and the doctor examined all of them once First the children were examined by the nurses and about two weeks later by the doctor who at that time was ignorant of the nurses results A standardized and structurized schedule including a manual was used by all examiners

The following aspects of the physical examinations were studied and compared somatic and motor development skin spine joints and feet The methods of classifying deviations are shown in Table 1

In addition the doctor s physical examination included auscultation of the heart measurement of blood pressure palpation of the thyroid gland and lymphnodes of the abdomen and in boys of the genital organs

The nurses physical examination included a vision and an auditory screening The methods are described elsewhere (15) and the results are only briefly reported here

Additional data of the children s health problems were registered with the help of written questionnaires to the parents and from interviews with their teachers These result will be published separately

Weight and height were plotted on standard curves for Swedish children (25) Signs of scoliosis were specifically looked for in the spine examination The children were observed from the front side and back while standing erect and bending forward the spine being flexed to approximately 60° (4 8)

Shoulder height asymmetry or prominence of the rib cage were noted Static scoliosis was excluded by compensation of unequal leg length with a plate under the shorter leg

Evaluation of the motor development was made by testing the coordination of fine and gross movements Each child was observed when walking normally tiptoeing walking on his heels and on the lateral borders of his feet (Fog s test) (9) and when jumping on one leg Results of the Fog s test revealing pronounced or unilateral supination pronation and extension or excessive grimacing were scored as abnormalities The testing of involuntary movements described by Prechtl (26) but modified (15) was used Fine movements were observed when the child in rapid succession and in proper order opposed the thumb to the fingers of the same hand threaded a string drew a circle rhomb and square Deviations in any of the described exercises classified the child as clumsy (Table 1)

The health problems or deviations from normal were classified as to their severity or functoinal importance in group 1 2 and 3 (Table 1) according to a method introduced by Kohler (17)

Deviations of groups and 3 were classified as significant or functionally important and the slight deviations of group 1 as insignificant or unimportant Health problems that had already been detected and were under observation or treatment before the actual investigation were defined as previously known

The nurses divided their children into two groups according to their need for a doctor s examination 1 Referral 2 Not referral The time needed to examine each child was recorded

RESULTS

An analysis of all physical health problems including visual and auditory defects of the 10-year-old children have been published earlier (15) The health problems of the 12 year-old children were found to be very much the same (Table 2)

All deviations disclosed in the physical examinations are presented in Figs 1 and 2 More than half of the children were found to have slight deviations from normal The nurses registered 104 more deviations than the doctor The doctor detected 35 deviations not observed by the nurses The nurses classified 29 (11%) of their deviations as functionally important the doctor 23 (14%)) of his The doctor didnot classify 8 of the nurses findings as significant 2 deviations in somatic development and 6 cases of suspected scoliosis The doctor noted 2 significant disorders not observed by one of the nurses Morbus Schlatter in a 12 year-old boy and allergic eczema in a 10 year-old girl It is possible that these children had less symptoms when the nurse made her examination three weeks earlier

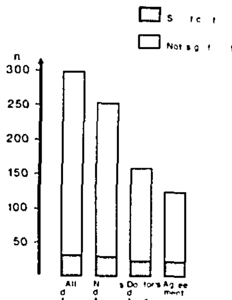


Fig. 1. Deviations detected in physical examination of 10 to 12 year old children.

Table 4. *A test for accuracy of the nurse's physical examination as a screening examination*

Nurse	Doctor: Detected significant health problems	Doctor: Not detected significant health problems	Total
Detected significant health problems	21	8	29
Not detected significant health problems	2	379	381
Total	23	387	410

Sensitivity (21×100)/23=91% Specificity (379×100)/387=98% Positive predictive value (21×100)/29=72% Youden Index (sensitivity + specificity − 100)=(91+98)−100=89%

Overweight (weight/height ≥+2 S.D.) was apparent in 5% of all the children and spine deformities in more than 10%. Of all children 1.5% had significant structural scoliosis. Slight disturbances in motor development were noted in 8% of the children, less frequent in the 12 year-olds (5%) than in the 10 year-olds (10%).

Altogether 8 significant disorders were newly detected by the doctor (Table 3). Half of them were structural scoliosis later confirmed by an orthopaedic specialist and put under close observation.

If the nurse's examination is regarded as a screening examination and the doctor's evaluation of significant health problems is considered as a diagnostic examination, the accuracy of the physical screening could be calculated according to Table 4. The specificity would be 98%, the sensitivity 91%, the posi-

Table 3. *Functionally important health problems observed by the doctor in the physical examination*

Diagnoses	Previously known (*n*)	Newly discovered (*n*)	Total (*n*)
Obesity	4	0	4
Asthma	1	0	1
Allergic rhinoconj	0	1	1
Aplasia of fibula	1	0	1
Ichtyosis	1	0	1
Allergic eczema	4	0	4
Foot mycosis	0	1	1
Scabies	0	1	1
Structural scoliosis	2	4	6
Mb Schlatter	1	1	2
Flat feet	1	0	1
Total	15	8	23

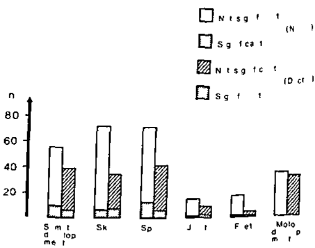


Fig. 2. Deviations from normal diagnosed by the nurse and the doctor in the physical screening of 410 10 to 12 year old children.

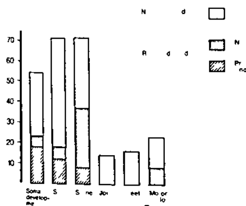


Fig 3 Referrals to the school physician by the nurses

tive predictive value 72% and the Youden index 89% (10)

The traditional auscultation and palpation examination was also made on the children by the school physician Two heart murmurs were noted both previously known and under treatment In addition two boys with phimosis were newly detected and referred for surgery

The nurses considered that their screening was sufficient as a physical appraisal in 80% of the children In the other 20% they considered that doctor s consultation was needed (Fig 3) Half of these children had health problems previously known and in need of a follow-up In the other half the nurses suspected deviations mostly in growth and development and of the spine

The school physician could not find any abnormality in half of the cases which were referred to her (Fig 4) The nurses and the physician spent an average of 5 min on each child Since the nurse measured weight and height and performed a vision screening at the same session the added examination did not take up much extra time Thus if the nurse is made responsible for the physical screening examination and takes care of 80% of the problems on her own the doctor would approximately save 80% of the time he usually spends on class-examinations

DISCUSSION

The purpose of physical examinations of school children is to reveal functionally important defects in need of treatment and to detect conditions where appropriate intervention can minimize or prevent functional impairment Deviations in growth and development spine deformities and allergic diseases are examples of health problems which often are asymptomatic or neglected in prepubertal age if they are not specifically looked for in health controls Thanks to an efficient preschool health programme in Sweden handicaps and chronic disorders as e g neurological diseases and congenital heart diseases are fully controlled In the present investigation 8 functionally important health problems were detected by simply looking at the child while the traditional doctor s examination with auscultation and palpation revealed only 2 cases of phimosis which should have been discovered at the school entrance This study indicates that examinations of the children by a nurse only would be quite sufficient as a screening with high sensitivity and specificity regarding functionally important deviations

The role and the skill of the Swedish school nurses seem to be underestimated Their medical training is solid and comprehensive and qualifies them to take advanced medical responsibilities in health surveillance and primary

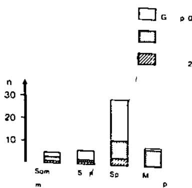


Fig 4 The school physician s classification of newly detected health problems referred by the school nurses

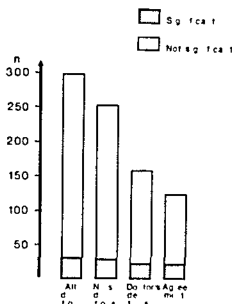


Fig 1 Deviations detected in physical examination of 10 to 12 year old children

Table 4 *A test for accuracy of the nurse's physical examination as a screening examination*

Nurse	Doctor: Detected significant health problems	Doctor: Not detected significant health problems	Total
Detected significant health problems	21	8	29
Not detected significant health problems	2	379	381
Total	23	387	410

Sensitivity (21×100)/23=91% Specificity (379×100)/387=98% Positive predictive value (21×100)/29=[illegible]% Youden Index (sensitivity + specificity − 100)=(91+98)−100=89%

Overweight (weight/height ≥+ 2 S D) was apparent in 5% of all the children and spine deformities in more than 10% Of all children 1 5% had significant structural scoliosis Slight disturbances in motor development were noted in 8% of the children less frequent in the 12 year-olds (5%) than in the 10 year olds (10%)

Altogether 8 significant disorders were newly detected by the doctor (Table 3) Half of them were structural scoliosis later confirmed by an orthopaedic specialist and put under close observation

If the nurse's examination is regarded as a screening examination and the doctor's evaluation of significant health problems is considered as a diagnostic examination the accuracy of the physical screening could be calculated according to Table 4 The specificity would be 98% the sensitivity 91% the post

Table 3 *Functionally important health problems observed by the doctor in the physical examination*

Diagnoses	Previously known (*n*)	Newly discovered (*n*)	Total (*n*)
Obesity	4	0	4
Asthma	1	0	1
Allergic rhinoconj	0	1	1
Aplasia of fibula	1	0	1
Ichtyosis	1	0	1
Allergic eczema	4	0	4
Foot mycosis	0	1	1
Scabies	0	1	1
Structural scoliosis	2	4	6
Mb Schlatter	1	1	2
Flat feet	1	0	1
Total	15	8	23

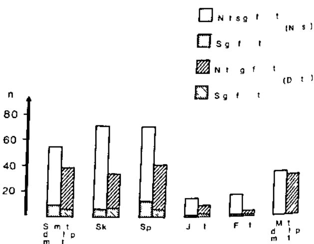


Fig 2 Deviations from normal diagnosed by the nurse and the doctor in the physical screening of 410 10 to 12 year old children

11 Grant M W W Fearnow R G Herbertson C L M & Henderson A L Health screening in school age children The physician and paramedical personnel *Am J Dis Child 125* 5 0 1973
1? Greenfield S Komaroff L Pass T M Anderson H & Nessim S Efficiency and cost of primary care by nurses and physician assistants *N Engl J Med 298* 305 1978
13 Haggerty R J Roghmann K J & Pless I B *Child health and the community* Wiley New York 1975
14 Helve A Skolhalsovården en del av folkhalsoarbetet i Finland *Nord Med 91* 85 1976
15 Kornfalt R & Kohler L Physical health of ten year-old children An epidemiological study of school children and a follow up of previous health care *Acta Paediatr Scand 67* 481 1978
16 Kornfalt R Malmberg M & Roslund I Report of a school health team in an elementary school In preparation
17 Kohler L Physical health of 7 year old children An epidemiological study of school entrants and a comparison with their preschool health *Acta Paediatr Scand 66* ?97 1977
18 Kohler L Physical mass examinations in the school health service *Acta Paediatr Scand 66* 307 1977
19 Kohler L & Andersson I Case report of a Swedish school health clinic *Acta Paediatr Scand 68* Suppl ?75 1979
?0 *Problems of children of school age (10–13 years)* Report on a working group WHO Copenhagen 1976
?1 Sells C J & May E A Scoliosis screening *Am J Nursing 74* 60 1974
?? Silver H K Igoe J B & McAtee P R The school nurse practitioner Providing improved health care to children *Pediatrics 58* 580 1976
23 Silver H K Ford L C & Day L R The pediatric nurse practitioner programme *JAMA 240* ?98 1968
?4 *Skolhalsovard* Betankande av 1974 års skolhalsovårdsutredning SOU 1976 46
25 Taranger J The somatic development of children in a Swedish urban community A prospective longitudinal study *Acta Paediatr Scand* Suppl 258 1976
?6 Touwen B C & Prechtl F R The neurological examination of the child with minor dysfunction *Clin Dev Med 38* Spastics International Medical Publications 1970
?7 Utbildningsdepartementet *Skolhalso a dsutredningens enkatundersokningar till berorda befattningshavare* Ds U 1976 3 Gotab Stockholm 1976
?8 Willner S *Factors contributing to structural scoliosis* Thesis Studentlitteratur Lund 1972
?9 Yankauer A Allied health workers in pediatrics *Pediatrics 41* 1031 1968
30 Yankauer A Child health supervision is it worth it? *Pediatrics 52* ?7? 1973
31 Yankauer A & Lawrence R A A study of periodic school physical examinations *Am J Public Health 51* 153? 1961

Submitted March ? 1979
Accepted April 21 1979

(R K) Department of Pediatrics
University Hospital
S ?21 85 Lund
Sweden

care which they often do compared to nurses in many other countries Their qualifications are comparable to university educated American nurse practitioners and health assistants who act as primary health care providers at preschool and school age in many parts of USA and have proved as competent in health care as doctors or even better (7 8 11 12 22 23 29) The scoliosis screening in some school districts was delegated to nurses and resulted in a considerable increase of diagnosed cases of idiopathic scoliosis (1 21)

The Swedish school nurses should be perfectly capable of handling some of the doctor s traditional work in school health

From the present investigation it seems reasonable to suggest that physical screening of 10 to 12 year old school children should include

1 Assessment of the child s growth

2 Examination of the spine including the bending forward test for detection of scoliosis

3 Inspection of the skin

In connectoin with the examination anamnestical data concerning the child s health and well being should be collected from the children their parents and teachers Vision and hearing screening should also be made

The school entrance health examination should probably still be undertaken by the doctor mainly because it would give him/her opportunity to establish a good contact with the child and its parents

A rational economic use of medical resources is of course of utmost importance in health care planning The methods used in this study reduce the costs for the school health without diminishing the efficiency There is a considerable difference in salary between a doctor and a nurse US$34/hour compared to US$9/hour for the nurse (including employer s charge 44%, January 1979) The cost per child for a doctor s examination is approximately US$2 8 and for a nurse s examination US $0 75 (5 min/child or 12 children/hour) If the nurse examines the children first for height and weight and visual acuity and assists the doctor when he makes his physical examination as is usual in Sweden the costs per 100 children will amount to US$150 (nurse) + US$280 (doctor) = US$430 If the doctor examines only 20 children the costs will be US$90 (nurse) + US$56 (doctor) a reduction of costs by 60–70%

Thus it is possible without requesting more resources to utilize the expensive school doctor for tasks for which his specialized skill is needed as medical care for acute and chronic illnesses for health education to children families and teachers and for a deeper concern about social and psycho social aspects of the child s wellbeing which should also be covered by the health surveillance in school

The school health service could obviously be a useful and highly estimated service All children go to school are easily accessible and through them also their homes and families Thereby it is possible and also necessary to take a major responsibility for the children s health

REFERENCES

1 Baker E A & Zangger B School screening for idiopathic scoliosis *Am J Nursing 70* 767 1970

2 Brundtland G H Skolen – sentralt angreppspunkt i forebyggende helsearbeid *In* Ø Larsen (ed) *Forebyggende medisin* Universitetsforlaget Oslo 1975 p 67

3 *Child Health Report of a European symposium* WHO Copenhagen 1970

4 Dunn B H Hakala M W & McGee M E Scoliosis screening *Pediatrics 61* 794 1978

5 Fek K & Fellenius G *Skolhalsovard en handbok* Liber laromedel/Utbildningsförlaget Stockholm 1976

6 *Evaluation of School Health Programmes* Report on a working group WHO Copenhagen 1978

7 Fine L L The pediatric practice of the child health associate *Am J Dis Child 131* 634 1977

8 Foye H Chamberlin R & Charney E Content and emphasis of well child visits Experienced nurse practitioners vs pediatricians *Am J Dis Child 131* 794 1977

9 Fog E & Fog M Cerebral inhibition examined by associated movements *Clin Dev Med* (Heineman London) *10* 52 1963

10 Frankenburg W K Pediatric screening *Adv Pediatr 20* 149 1973

Acta Pædiatr Scand 68 887–891 1979

SELF CONTROL WITH URINALYSIS IN JUVENILE DIABETES

J LUDVIGSSON and P G SVENSSON

From the Department of Paediatrics University Hospital and the Department of Sociology Linkoping University Linkoping Sweden

ABSTRACT Ludvigsson J and Svensson P G (Department of Paediatrics University Hospital and Department of Sociology Linkoping University Linkoping Sweden) Self-control with urinalysis in juvenile diabetes Acta Paediatr Scand 68 887 1979 —Urinary glucose excretion reflects the blood glucose levels and is therefore recommended and used as a relevant and practical method for self-control in juvenile diabetes The purpose of this study was to estimate the attitudes of diabetic children and their parents towards such daily urinalysis In 1975 69 juvenile diabetics 6–18 years old and their parents were studied and three years later another 69 patients were added Standardized interviews and questionnaires were used Only 3 out of 138 patients refused to test their urine regularly and to write down their results in the diary The results indicate that a great majority of the patients and the parents easily accept the self testing method and regard it as a valuable tool in the management of the disease Almost nobody experienced the urine tests as a psychological problem

KEY WORDS Self control urinalysis juvenile diabetes attitudes

Diabetic children when in metabolic balance near normoglycemia feel subjectively well grow normally get normal pubertal development (14) and have a better defence mechanism against infections (1 3) Most evidence supports the view that in such children late vascular complications are at least postponed (4 7) Insulin diet and physical exercise are the classical cornerstones to achieve such balance but there is less agreement on the methods for evaluating the results of the treatment Determinations of blood glucose levels or urinary glucose excretion in connection with sporadic visits to a diabetic clinic give only snapshots of reality Haemoglobin A_1 reflects the longterm blood glucose levels better (5 8) but only in retrospect and hence the information obtained through haemoglobin A_1 determinations comes too late and is of limited help in the regulation of the actual daily blood glucose variation There is a need for a reliable and valid parameter giving day to day information about the patient s metabolic balance For practical and psychological reasons one cannot recommend diabetic children to have their blood glucose determined several times every day (2 17) However in patients with a normal renal threshold for glucose urinary glucose excretion reflects the blood glucose levels (6 10 13 15) and has therefore been recommended and used as a relevant and practical method for determination of the metabolic control (10 11 18) However many physicians have been reluctant to accept this principle of daily urinalysis at home Urinalysis is said to be unreliable to in

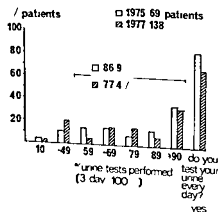


Fig 1 Frequency of urine tests performed

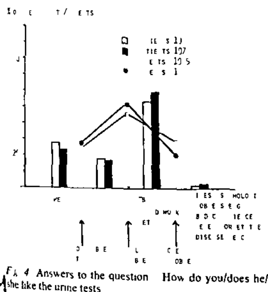


Fig 4 Answers to the question How do you/does he/she like the urine tests

well as to age at onset ($p<0.05$) A high frequency was positively correlated to the number of tests with no or minimal (<1%) glucosuria ($p<0.01$) i e to better metabolic control (10)

Two years later 3 patients all teenagers out of 138 refused to test their urine regularly and to write down the results in the diary (Fig 1) although they declared that they did test their urine sporadically especially when they did not feel well There was no significant change in test frequencies between 1975 and 1977 although the proportion of patients who tested their urine <50% of expected number had increased parallell to a decreasing proportion of patients who said they tested their urine every day (Fig 1) All patients who tested their urine <50% of expected number were more than 12 years old both 1975 and 1977 Beside the drop in test frequency after the first two years there was no further decrease related to increasing duration (Fig 2)

In 1975 the attitudes towards the urinalysis were predominantly positive in 56 patients (83 6%) The patients motivation for the treatment was positively correlated to the motivation of their parents ($p<0.01$) A positive attitude towards the team at the diabetic clinic was related to a good motivation for the treatment both among patients ($p<0.01$) and parents ($p<0.05$)

The attitude towards different parts of the treatment among the 121 patients interviewed in 1978 was compared to that of the 69 in 1975 in the answers to a question whether there was anything in the treatment that the patients did not like particularly (Fig 3) Although the proportion of patients who did not like the urinary tests had increased it is rather small and the parent s interpretation of their children s reaction is still more positive with regard to the urinary tests

On the question How do you like the urine tests? almost nobody experienced the urine tests as a heavy burden leading to bad conscience psychological problems etc However about half of the patients think the tests are rather boring (Fig 4) The remaining patients either liked the tests well or accepted them This pattern is in good agreement with the parents estimate of the opinion of their children Although many patients and parents thus think the urinary tests are boring a great majority of both patients and parents feel that the urine tests are of value and useful (Fig 5) The practical usefulness of the urine testing was acknowledged by 74 6% of the parents in 1975 and by 94 8% in 1978 The proportion of parents who felt it important to test the chil

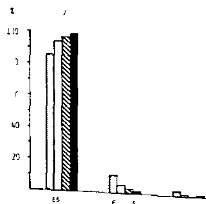


Fig 5 Answers to the question Do you think the urine tests are of value? □ patients 1975 □ patients 1978 ▧ parents 1975 ■ parents 1978

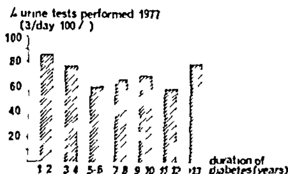


Fig 2 Frequency of urine tests performed by the patients in relation to the duration of their disease

crease the anxiety of the patient and the fixation to the disease and to be unacceptable by the patients (12) The purpose of this study was for the first to determine the urine test frequency and correlate to some parameters and for the second to estimate the attitudes of diabetic children and their parents towards daily urinalysis at home and to correlate these attitudes to the actual urinalysis situation

MATERIAL

Every diabetic child or adolescent in the hospital region is controlled at the diabetic clinic of the pediatric department The patients are treated with insulin once or twice daily regulated diet and regular physical exercise Since 1971 they have all been recommended to test single voided urine specimens at home with the Clinitest 2 drops method at least 3 times daily (before breakfast before dinner in the afternoon and before they go to bed) and in addition sometimes before lunch if practically possible The urine is tested for ketone bodies by Ketostix when the patients have acute infections are ill do not feel well or have 2% glucose in the urine in repeated specimens The results of the urine tests are registered in a special diary which is analysed by the nurse and doctor at the hospital visit every 2–3 months

In 1975 69 juvenile diabetics 6–18 years old were studied Their age at onset of diabetes varied between 1–14 (6 4±3 6) years and the duration of the disease between 3–17 (7 6±3 3) years Three years later another 69 patients were added The age of the 138 patients varied between 1–21 years age at onset of diabetes from 1 1… (7 3±4 0) years and duration of diabetes from 1 1… (5 7±3 6) years

METHODS

In 1975 the attitudes towards the treatment were studied in two ways in all but two patients who were less than 7 years old A standardized interview was constructed containing 52 questions related to the following items Content of diet regularity of diet physical exercise urinalysis at home insulin injections visits to the diabetic clinic and regularity of daily activities In addition a special attitude test was constructed which consisted of 34 provocative statements related to the same items (10) Each field was covered by negative and positive statements which the patients had to accept or reject whereby their attitudes could be classified from very negative to very positive The special test was used as a control of the validity of the interviews The result of the special attitude test used in 1975 was well correlated to the result of the interviews ($r=0.78$ $p<0.001$)

Three years later we used a questionnaire with the same questions as the standardized interviews No answer was returned from 13 teenagers and 4 children were too young

All diaries were collected and the frequency of performed urinary tests was counted

RESULTS

Out of the 69 patients all but two teenagers tested their urine regularly during 1975 (Fig 1) The frequency of testing was negatively correlated to onset before 1970 ($p<0.05$) as

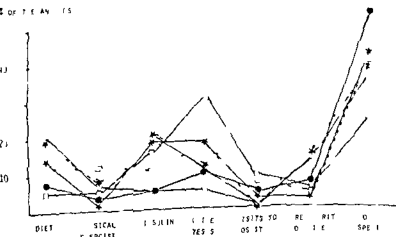


Fig 3 Answer to the question Is there anything in the treatment you dislike especially much? ★—★ patients 1975 ●—● patients 1978 ○—○ parents 1975 □—□ parents 1978 ★ ★ (=1975) and □ □ (=1978) shows what the parents believe(s) their children dislike most

controlled diabetic patients *Am J Med Sci* 263 451 1972

2 Bernstein R K Protein as the principal scource of dietary carbohydrate for the tight control of glycemic levels in juvenile onset of diabetes Abstract *4th International Beilinson Symposium on Nutrition and the diabetic child* Israel 1978

3 Bybee J D & Rogers D E The phagocytic activity of polymorphonuclear leucocytes obtained from patients with diabetes mellitus *J Lab Clin Med* 61 1 1964

4 Cahill G F Etzwiler D D & Freinkel N Control and diabetes *N Engl J Med* 294 1004 1976

5 Ditzel J & Kjaegaard J J Haemoglobin A concentrations after initial treatment for newly discovered diabetes *B Med J* 1 741 1978

6 Forman B H Goldstein P S & Genel M Management of juvenile diabetes mellitus usefulness of 4-hours fractional quantitative urine glucose *Pediatrics* 53 57 1974

7 Jackson R L Juvenile diabetes *In* W J Malaisse & J Pirart (eds) *Diabetes* Excerpta Medica Amsterdam 1974 p 5 0

8 Koenig R J Peterson C M Jones R L Saudek C Lehrman M & Cerami A Correlation of glucose regulation and haemoglobin A_1 in diabetes mellitus *N Engl J Med* 295 417 1976

9 Lanoe R Thibult N Eschwege E Soria J Soria C & Tchobroutsky G Glycosylated haemoglobin concentrations and Clinitest results in insulin dependent diabetes *Lancet* II 1156 1977

10 Ludvigsson J *Metabolic control in juvenile diabetes mellitus* The influence of some clinical biochemical and socio-psychological factors Linkoping University Medical Dissertations no 42 Zata tryckeriema Linkoping 1976

11 Ludvigsson J Hemmakontroll av urinen i behandlingen av juvenil diabetes mellitus *In* O Aagenaes (ed) *Diabetes i barnearena* (Childhood diabetes) Oslo 1976

12 Malone J I Hellrung J M Malphus F W Rosenbloom A L Gric A & Weber F T Good diabetic control—a study in mass delusion *J Pediatr* 88 943 1976

13 Molnar G D Gastineau C F Rosevear J W & Moxness K E Quantitative aspects of labile diabetes *Diabetes* 14 279 1965

14 Petersen H D Korsgaard B Deckert T & Nielsen E Growth body weight and insulin requirement in diabetic children *Acta Paediatr Scand* 67 453 1978

15 Service F J Monar G D & Taylor W F Urine glucose analyses during continuous blood glucose monitoring *JAMA* 222 294 1973

16 Topper E Doron M Karp M & Laron Z *The relationship between glycosylated hemoglobin and postprandial blood glucose in insulindependent juvenile diabetics* Internat Diabetes Study Group Israel 1978

17 Walford S Gale E A M Allison S P & Tattersall R B A pilot study of home blood glucose monitoring Abstract *4th International Beilinson Symposium on Nutrition and the diabetic child* Israel 1978

18 Weber B Validity and practicability of glucosuria selfcontrol in juvenile diabetics *In The balance of diabetes in juveniles* International Beilinson Symposium Israel 1975

Submitted March 12 1979
Accepted May 21 1979

(J L) Department of Paediatrics
University Hospital
S 581 85 Linkoping
Sweden

dren s urine even in periods when the child feels well had also increased from 71% in 1975 to 95% in 1978 The growing feeling of usefulness of the diary for the patients and the parents themselves is also reflected by the answer to a question on the possibility of writing down a false or guessed figure instead of actually making the test In 1975 19% thought this might happen but only 5% in 1978

DISCUSSION

Several studies support the reliability and validity of daily determinations of glucose in single voided urine specimens through the Clinitest 2 drops method and this has been accepted as a relevant and useful method in the management of juvenile diabetes (6 9 10 11 16 18) The three times recommended are chosen with regard to the school but of course it is sometimes necessary to add a urine test before lunch to control the morning glucosuria

This study shows that it is possible to get an overwhelming majority of patients to test their urine regularly for several years A few teenagers refused totally while another group of teenagers tested their urine less frequently but still on an average almost once a day The cooperation was quite as good as with regard to other parts of the treatment The negative correlation between test frequency and onset before 1970 when urine tests were not recommended indicates that in order to get a good cooperation it is important to inform both patients and parents about the importance of the urine tests already at the onset of the disease The negative correlation to higher age at onset reflects the increasing problems to get teenagers to cooperate The correlation between high frequency and better metabolic control probably means both that urine tests contribute to better metabolic control and that it is more acceptable for those with a good metabolic control to test their urine

The results obtained at the interviews in 1975 were validated through comparison with a special attitude test The reliability and validity of attitudes assessment tests are nevertheless uncertain and the results must be interpreted with caution Furthermore in 1978 we got no answer from 13 teenagers whose attitudes reasonably were predominantly negative Still with these facts in mind the results strongly indicate that without strict rules but with repeated information we may not have been able to make the urine testing pleasant However although the patients disliked urine tests more than any other part of the treatment a great majority of the patients and the parents found the urine tests valuable and they accepted the urine tests as one of several necessary cornerstones in the treatment Almost nobody experienced the urine tests as a psychological problem although it happens occasionally that we have to advise some patients to test less frequently when a too strong fixation to the disease seems to be developing Our main impression is that daily home testing of the urine predominantly means psychological advantages The patients or parents get the possibility to manage the treatment they learn the effects of diet physical exercise insulin infections etc and can avoid acute complications Thus the urine tests contribute to increasing knowledge increasing confidence and independence a more positive attitude to other parts of the treatment and as a consequence a better metabolic control The facts that urine tests during period are rejected by some teenagers and badly informed parents or may be taken too serious by a few others are no justifications for rejecting this important part of the treatment of juvenile diabetes

ACKNOWLEDGEMENTS

This study has been supported by the Swedish Medical Research Council No B78 19X 04528 04A and the Swedish Diabetic Association

REFERENCES

1 Bagdade J Nielson K L & Bulger R J Reversible abnormalities in phagocytic function in poorly

Acta Pædiatr Scand 68 893–897 1979

DIAGNOSIS OF HIRSCHSPRUNG S DISEASE

M KEKOMAKI J RAPOLA and I LOUHIMO

From the Child en s Hospital Helsinki University Central Hospital Helsinki Finland

ABSTRACT Kekomaki M Rapola J and Louhimo I (The Children s Hospital Helsinki University Central Hospital Helsinki Finland) Diagnosis of Hirschsprung s disease Acta Paediatr Scand 68 893 1979 —A prospective study of the accuracy of various diagnostic methods used in the detection of Hirschsprung s disease (syn congenital intestinal aganglionosis CIA) in 60 consecutive infants and children was done during the period 1972–76 Every patient underwent a barium enema a rectal mucosal biopsy which was prepared for both the demonstration of ganglia and for the assessment of acetylcholinesterase activity (ACE) and anal manometry was performed In evaluating the clinical history special emphasis was placed on signs of neonatal ileus In the group of 10 patients with a definite diagnosis of CIA the results were almost uniform In the non CIA group the search for ganglia in biopsy material proved non confirmatory in nearly half of the cases studied due to the fact that specimens were taken too superficially The findings pertaining to ACE barium enema and the results of manometry were at variance or inconclusive of a final diagnosis in 10 16 and 22 % of the performed studies respectively The value given to neonatal history proved to be of the same order i e 0 % proved to be falsely positive

KEY WORDS Megacolon intestinal occlusion manometry acetylcholinesterase

Since the recognition of the absence of ganglion cells in the intrinsic nerve plexuses of the rectum as a *conditio sine qua non* of Hirschsprung s disease (syn congenital intestinal aganglionosis CIA) a search for ganglion cells in the myenteric and submucosal nerve plexuses of the terminal rectum has served as method of diagnosis in CIA Two factors limit the usefulness of rectal biopsy The taking of a too superficial biopsy does not always yield an adequate sample for the establishment of the presence of ganglion cells and a full thickness rectal biopsy carries the risk of complications An X ray examination of the rectum with a barium enema also has its limitations especially in short segment disease and also in higher lesions at an early age when megacolon has not yet developed (5 18) Therefore other indirect methods have been introduced in order to arrive at a tentative diagnosis of CIA These include the determination of the reflex functions of the internal sphincter of the rectum by anal tonometry (9 14) and a histochemical investigation of the distribution of acetylcholinesterase (ACE) in the muscularis mucosae and lamina propria of the rectal mucosa (11) We present here the results of investigations of 60 cases in which these techniques have been employed in the diagnosis of CIA

PATIENTS AND METHODS

Patients The material consisted of 60 consecutive patients with severe constipation examined at the Children s Hospital Helsinki University Central Hospital during the years 1972–76 All these patients were subjected to (1) barium enema (2) anal manometry and (3) suction or punch biopsy of the rectal mucosa The sex of the patients their age at the time of the investigation and the final clinical diagnosis whether CIA or non CIA are given in Fig 1 In operated cases the final diagnosis of CIA was established by a histologic examination of the resected bowel Symptoms and signs suggestive of intestinal obstruction (abdominal swelling marked constipation and/or vomiting meconium ileus and spontaneous perforation of the gut) were regarded as neonatal symptoms and signs suggestive of CIA (18)

Methods A barium enema without any preceding evacuation of the rectum was performed (17) Tension changes in the internal sphincter were recorded by means of a simple self-constructed anal tonometer (4) A decrease in the sphincteric tone ranging from 5 to 15 mmHg

Table 1 *Neonatal histor\ and results of in\ estigations of 10 patients treated operatn el) for CIA*
Positivity (+) signifies findings suggestive of CIA

	Patient							
No	Sex	Age (years)	Neonatal history	Barium enema	Mano metry	Search for ganglia	ACE	Note
1	♂	?/5?	Ileus	+	+	(•)	+	–
	♂	/5?	Ileus	+	( )	+	+	–
3	♂	1/1?	Ileus sepsis?	+	+	+	+	21 trisomy
4	♂	4/1?	Vomiting diarrhea	+	+	(•)	+	–
5	♀	9/1	Sepsis? Mal absorption?	+	+	+	+	–
6	♂	1	Sepsis	+	+	+	+	–
7	♂	1	Vomiting	+	+	(•)	+	–
8	♂	1	Ileus	+	–	+	+	–
9	♂	?	Vomiting sepsis	+	+	+	+	–
10	♀	11	Vomiting	+	+	+	+	–

Technical difficulties in manometry
• Amount of submucosa judged insufficient to allow a definite diagnosis

ures in the manometry 29 patients out of 50 had a completely normal pattern of findings combined with a normal neonatal history Of the remaining 21 7 had defecation difficulties during the immediate neonatal period as the only feature suggestive of CIA This group included 2 patients with a low (translevator) anorectal malformation 2 patients with idiopathic perforation of the colon 2 patients with minor defecation difficulties and one patient with a meconium plug syndrome

Eight patients in the non CIA group had one pathological finding as regards barium enema manometry or abnormal histology of the biopsy material (Table 2)

In 6 of the non CIA patients two or three diagnostic features out of the five were considered suggestive of CIA Their detailed distribution is given in Table 3

Table 2 *Sporadic findings suggestn e of CIA in 50 non CIA infants and children*

Technique of examination	Number of patients
Barium enema	3
Manometry	3
Search for ganglia	1
ACE	1
Total	8

DISCUSSION

Classically the diagnosis of CIA is based on a typical clinical history a barium enema and a full thickness rectal biopsy Manometry and the determination of the distribution of ACE in the rectal mucosa have been recommended in recent years as a screening procedure for patients prior to more extensive examinations Whatever the examination technique employed the value of the results obtained appears to be limited by the age and condition of the patient (7 13 16) However a more serious limitation is that all the techniques are prone to both technical failures and errors of interpretation

The main aim of our study was to examine the possibility that the diagnostic accuracy of CIA could be improved by the performance of mutually independent methods of investigation Referring to Table 4 we are able to answer this question in the affirmative

Of the 10 patients with CIA at least four abnormal findings out of five were considered to indicate CIA In fact discrepant findings were limited to only one case with a normal manometric tracing (Table 1)

The non CIA patients can be divided into two subgroups The first consists of patients (44/50) with none or only one finding sug

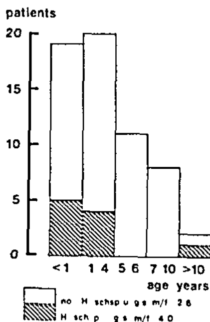


Fig 1 Age distribution and sex ratio of patients



Fig 2 Distribution and activity of ACE pathognomonic for CIA No counterstaining Thick intensely stained nerve fibers are present in the submucosa (*S*) and muscularis mucosae (*M*) Thin positive fibres are seen in the lamina propria (*l*) ×200

was interpreted as a normal response Lack of internal sphincter inhibition or a slight increase in pressure were interpreted as abnormal Resting pressure was not routinely recorded To establish the reliability of the results manometry was repeated 4–5 times during the same hypnosis (Ketalar* Parke Davis & Co Pontypool Mon U K 4–5 mg/kg b wt)

At least two specimens containing sufficient submucosa were taken at the level of anal valves (pectinate line 2) *One of the samples was spread on a piece of* absorbent paper with the mucosal surface upwards and fixed in formalin Serial sections were used to search for submucosal ganglion cells Another specimen was frozen rapidly in liquid nitrogen Sections were stained for the histochemical determination of ACE (11) The presence of ganglia was determined in those paraffin sections which contained sufficiently submucosa The sections stained for ACE were classified in three groups (1) In normal mucosa there was faint and diffuse staining of the muscularis mucosae and a few thin positively staining nerve fibres at the border of the muscularis mucosae and submucosa (2) The presence of ACE was considered positive (=*suggestive of CIA) when there were many strongly* positively staining nerve fibre bundles in the muscularis mucosae and numerous positively staining nerve fibres in *the lamina propria* (Fig 2) (3) In a few cases an indeterminate pattern was found with occasional positively staining fibres in the lamina propria This finding has been marked ± in the tables

RESULTS

Patients with CIA

The uniformity of the findings as regards the 10 patients with CIA was impressive (Table 1) In particular the barium enema results and the determinations of ACE were uniformly in agreement with the final diagnosis All histological specimens were found to be aganglionic However in one patient the result of the manometry was interpreted as being normal at repeated determinations

Positive neonatal histories were found in all the patients studied with CIA *In none* of the cases was this merely limited to mild coprostasis or abdominal swelling but there were clear signs of intestinal obstruction necessitating hospitalization during the immediate neonatal period

Patients with non CIA

Disregarding equivocal or negative findings due to obviously faulty technique such as biopsies too superficial for the search of ganglia to be made and purely instrumental fail

Table 1 *Neonatal history and results of investigations of 10 patients treated operatively for CIA*
Positivity (+) signifies findings suggestive of CIA

Patient								
No	Sex	Age (years)	Neonatal history	Barium enema	Mano metry	Search for ganglia	ACE	Note
1	♂	/5	Ileus	+	+	(*)	+	–
	♂	?/5?	Ileus	+	( )	+	+	–
3	♂	1/1?	Ileus sepsis?	+	+	+	+	21 trisomy
4	♂	4/1	Vomiting diarrhea	+	+	(*)	+	–
5	♀	9/1	Sepsis? Mal absorption?	+	+	+	+	–
6	♂	1	Sepsis	+	+	+	+	–
7	♂	1	Vomiting	+	+	(*)	+	–
8	♂	1	Ileus	+	–	+	+	–
9	♂	2	Vomiting sepsis	+	+	+	+	–
10	♀	11	Vomiting	+	+	+	+	–

Technical difficulties in manometry
* Amount of submucosa judged insufficient to allow a definite diagnosis

ures in the manometry 29 patients out of 50 had a completely normal pattern of findings combined with a normal neonatal history Of the remaining 21 7 had defecation difficulties during the immediate neonatal period as the only feature suggestive of CIA This group included 2 patients with a low (translevator) anorectal malformation 2 patients with idiopathic perforation of the colon 2 patients with minor defecation difficulties and one patient with a meconium plug syndrome

Eight patients in the non CIA group had one pathological finding as regards barium enema manometry or abnormal histology of the biopsy material (Table 2)

In 6 of the non CIA patients two or three diagnostic features out of the five were considered suggestive of CIA Their detailed distribution is given in Table 3

Table 2 *Sporadic findings suggestive of CIA in 50 non CIA infants and children*

Technique of examination	Number of patients
Barium enema	3
Manometry	3
Search for ganglia	1
ACE	1
Total	8

DISCUSSION

Classically the diagnosis of CIA is based on a typical clinical history a barium enema and a full thickness rectal biopsy Manometry and the determination of the distribution of ACE in the rectal mucosa have been recommended in recent years as a screening procedure for patients prior to more extensive examinations Whatever the examination technique employed the value of the results obtained appears to be limited by the age and condition of the patient (7 13 16) However a more serious limitation is that all the techniques are prone to both technical failures and errors of interpretation

The main aim of our study was to examine the possibility that the diagnostic accuracy of CIA could be improved by the performance of mutually independent methods of investigation Referring to Table 4 we are able to answer this question in the affirmative

Of the 10 patients with CIA at least four abnormal findings out of five were considered to indicate CIA In fact discrepant findings were limited to only one case with a normal manometric tracing (Table 1)

The non CIA patients can be divided into two subgroups The first consists of patients (44/50) with none or only one finding sug

Table 3. *Clustering of features suggestive of CIA in six patients from non-CIA group*
Positivity (+) denotes findings suggestive of CIA

	Patient							
No.	Sex	Age (years)	Neonatal history	Barium enema	Manometry	Search for ganglia	ACE	Note
1	♀	1/12	Normal	+	+	(+)	+	Congenital hypothyroidism, no symptoms
2	♂	2/12	Meteorismus	+	−	−	±	No symptoms
3	♀	3/12	Defecation difficulties	+	(−)	+	−	No symptoms
4	♀	6/12	Normal	+	−	−	±	21-trisomy
5	♂	1	Defecation difficulties	−	(+)	(+)	+	Uses laxatives irregularly
6	♂	8	Normal	+	−	−	±	Epidermolysis bullosa, anal fissure

For footnotes, see Table 1.

gestive of CIA. In the light of our experience these patients may be treated conservatively.

The second subgroup containing 6 infants and children with two or three diagnostic features indicating CIA, constitute a diagnostic dilemma (Table 3). The diversity of the findings may reflect the inclusion of a few cases with an ultrashort aganglionotic segment. It is therefore our present practice to stage therapy according to the clinical picture in patients with discrepant findings: if conservative treatment fails, anorectal myectomy is performed both for diagnostic and therapeutic purposes (10–12).

The percentage of biopsies taken too superficially for proper evaluation is much higher than that reported by Campbell & Noblett (3), for example. This may reflect our wish not to perform too deep biopsies, especially in an asymptomatic neonate. Despite our carefulness we saw one serious complication in this series, a rectal perforation in a neonate occurring during suction biopsy. Significant and even lethal bleeding can complicate a punch biopsy (13–15), though more rarely than in the case of open surgical biopsy.

In our experience the results obtained by manometry appeared to be compatible with those reported by Aaronson & Nixon (1). However, to limit the technical pitfalls in manometry it seems to be essential to have balloon systems of different sizes at one's disposal, as also suggested by El Shafie et al. (4).

The determination of acetylcholinesterase in the rectal mucosa is probably the most significant advancement in the diagnosis of CIA. Meier-Ruge et al. (11) are credited for the introduction of ACE determinations in clinical practice. Also in this series ACE determinations proved to be a method of great value. However, its specificity was somewhat reduced by the finding of an intermediate form of ACE distribution in three cases (Table 3).

Table 4. *Distribution of symptoms and clinical findings in 60 infants and children with suspected CIA*

The features evaluated are:
signs of neonatal occlusion,
absence of ganglia in rectal wall,
ACE in rectal lamina propria,
long anal canal in barium enema, and
lack of reflex relaxation of the internal sphincter in manometry

Number of positive symptoms and findings	Number of patients	Final clinical diagnosis
5	5	CIA
4	5	CIA
3	3	non-CIA
2	3	non-CIA
1	15	non-CIA
0	29	non-CIA

Otherwise our results are in agreement with the original observations of Meier Ruge et al Series with discordant results have also been published (6 19) However in a recent ap praisal of a large series the determination of ACE was found to be more reliable and con sistent than any of the other methods available for the diagnosis of CIA (8)

Consequently the two latest non invasive techniques employed in the diagnosis of CIA manometry and ACE seem to be of equal value to the classic ones They are recom mended for routine use at least in borderline cases

ACKNOWLEDGEMENT

This study was supported in part by grants from the Sigrid Juselius and Paulo Foundations Helsinki Finland

REFERENCES

1 Aaronson I & Nixon H H A clinical evaluation of anorectal pressure studies in the diagnosis of Hirsch sprung s disease *Gut 13* 138 197
Aldridge R I & Campbell P E Ganglion cell distribution in the normal rectum and anal canal A basis for the diagnosis of Hirschsprung s disease by anorectal biopsy *J Pediatr Surg 3* 475 1968
3 Campbell P E & Noblett H R Experience with rectal suction biopsy in the diagnosis of Hirsch sprung s disease *J Pediat Surg 4* 410 1969
4 El Shafie M Suzuki H Schnaufer L Haller J A & White J J A simplified method of ano-rectal manometry for wider clinical application *J Pediat Surg 7* 30 197
5 Ehrenpreis T Megacolon in the newborn A clinical and roentgenological study with special regard to the pathogenesis *Acta Chir Scand 94 Suppl 112* 1946
6 Garrett J R Howard E R & Nixon H H Histo-chemical diagnosis of Hirschsprung s disease *Lancet II* 436 1969
7 Holschneider A M Kellner E Streibl P & Sippell W G The development of anorectal con tinen e and its significance in the diagnosis of Hirsch sprung s disease *J Pediatr Surg 11* 151 1976
8 Lake B D Puri P Nixon H H & Claireaux A E Hirschsprung s disease An appraisal of histo-chemically demonstrated acetylcholinesterase ac tivity in suction rectal biopsy specimens as an aid to diagnosis *Arch Pathol Lab Med 10? ?44* 1978
9 Lawson J O N & Nixon H H Anal canal pres sures in the diagnosis of Hirschsprung s disease *J Pediatr Surg 2* 544 1967
10 Lynn H B Personal experience with rectal myec tomy in the treatment of selected cases of aganglionic megacolon *Z Kinderchir 5* Suppl *98* 1968
11 Meier Ruge W Lutterbeck P M Herzog B Mor ger R Moser R & Scharli A Acetylcholines terase activity in suction biopsies of the rectum in the diagnosis of Hirschsprung s disease *J Pediatr Surg 7* 11 197_
1? Nissan S Bar Maor J A & Levy E Anorectal myomectomy in the treatment of short segment Hirschsprung s disease *Ann Surg 170* 969 1969
13 Pease P W Corkery J J & Cameron A H Diagnosis of Hirschsprung s disease by punch biopsy of rectum *Arch Dis Child 51* 541 1976
14 Schnaufer L Talbert J L Haller J A Reid N C R W Tabon F & Schuster M M Differen tial sphincteric studies in the diagnosis of ano-rectal disorders of childhood *J Pediat Surg 2* 538 1967
15 Shandling B & Auldist A W Punch biopsy of the rectum for the diagnosis of Hirschsprung s disease *J Pediatr Surg 7* 386 1968
16 Smith B Pre and postnatal development of the ganglion cells of the rectum and its surgical implica tions *J Pediatr Surg 3* 386 1968
17 Swenson O Neuhauser E B D & Picket L K New concepts of etiology diagnosis and treatment of congenital megacolon *Pediatrics 4* _01 1949
18 Swenson C Sherman J O & Fisher J H Diag nosis of congenital megacolon an analysis of 501 pa tients *J Pediatr Surg 8* 587 1973
19 Trigg P H Belin R Haberkorn S Long W J Nixon H H Plaschkes J Spitz L & Willital G H Experience with a cholinesterase histo-chemical technique for rectal suction biopsies in the diagnosis of Hirschsprung s disease *J Clin Pathol 27* _07 1974

Submitted Nov 13 1978
Accepted April 11 1979

(M K) Department of Paediatrics
Turku University Central Hospital
SF 05 0 Turku
Finland

Table 3 *Clustering of features suggestive of CIA in six patients from non CIA group*
Positivity (+) denotes findings suggestive of CIA

	Patient							
No	Sex	Age (years)	Neonatal history	Barium enema	Manometry	Search for ganglia	ACE	Note
1	♀	1/12	Normal	+	+	(*)	+	Congenital hypothyroidism no symptoms
2	♂	2/12	Meteorismus	+	–	–	±	No symptoms
3	♀	3/12	Defecation difficulties	+	( )	+	–	No symptoms
4	♀	6/12	Normal	+	–	–	±	21 trisomy
5	♂	1	Defecation difficulties	–	( )	(*)	+	Uses laxatives irregularly
6	♂	8	Normal	+	–	–	±	Epidermolysis bullosa anal fissure

For footnotes see Table 1

gestive of CIA In the light of our experience these patients may be treated conservatively

The second subgroup containing 6 infants and children with two or three diagnostic features indicating CIA constitute a diagnostic dilemma (Table 3) The diversity of the findings may reflect the inclusion of a few cases with an ultrashort aganglionotic segment It is therefore our present practice to stage therapy according to the clinical picture in patients with discrepant findings if conservative treatment fails anorectal myectomy is performed both for diagnostic and therapeutic purposes (10 12)

The percentage of biopsies taken too superficially for proper evaluation is much higher than that reported by Campbell & Noblett (3) for example This may reflect our wish not to perform too deep biopsies especially in an asymptomatic neonate Despite our carefulness we saw one serious complication in this series a rectal perforation in a neonate occurring during suction biopsy Significant and even lethal bleeding can complicate a punch biopsy (13 15) though more rarely than in the case of open surgical biopsy

In our experience the results obtained by manometry appeared to be compatible with those reported by Aaronson & Nixon (1) However to limit the technical pitfalls in manometry it seems to be essential to have balloon systems of different sizes at one s disposal as also suggested by El Shafie et al (4)

The determination of acetylcholinesterase in the rectal mucosa is probably the most significant advancement in the diagnosis of CIA Meier Ruge et al (11) are credited for the introduction of ACE determinations in clinical practice Also in this series ACE determinations proved to be a method of great value However its specificity was somewhat reduced by the finding of an intermediate form of ACE distribution in three cases (Table 3)

Table 4 *Distribution of symptoms and clinical findings in 60 infants and children with suspected CIA*

The features evaluated are
signs of neonatal occlusion
absence of ganglia in rectal wall
ACE in rectal lamina propria
long anal canal in barium enema and
lack of reflex relaxation of the internal sphincter in manometry

Number of positive symptoms and findings	Number of patients	Final clinical diagnosis
5	5	CIA
4	5	CIA
3	3	non CIA
2	3	non CIA
1	15	non-CIA
0	29	non-CIA

Otherwise our results are in agreement with the original observations of Meier Ruge et al Series with discordant results have also been published (6 19) However in a recent appraisal of a large series the determination of ACE was found to be more reliable and consistent than any of the other methods available for the diagnosis of CIA (8)

Consequently the two latest non invasive techniques employed in the diagnosis of CIA manometry and ACE seem to be of equal value to the classic ones They are recommended for routine use at least in borderline cases

ACKNOWLEDGEMENT

This study was supported in part by grants from the Sigrid Juselius and Paulo Foundations Helsinki Finland

REFERENCES

1 Aaronson I & Nixon H H A clinical evaluation of anorectal pressure studies in the diagnosis of Hirschsprung s disease *Gut 13* 138 197
2 Aldridge R I & Campbell P E Ganglion cell distribution in the normal rectum and anal canal A basis for the diagnosis of Hirschsprung s disease by anorectal biopsy *J Pediatr Surg 3* 475 1968
3 Campbell P E & Noblett H R Experience with rectal suction biopsy in the diagnosis of Hirschsprung s disease *J Pediatr Surg 4* 410 1969
4 El Shafie M Suzuki H Schnaufer L Haller J A & White J J A simplified method of anorectal manometry for wider clinical application *J Pediatr Surg 7* 30 1972
5 Ehrenpreis T Megacolon in the newborn A clinical and roentgenological study with special regard to the pathogenesis *Acta Chir Scand 94 Suppl 112* 1946
6 Garrett J R Howard E R & Nixon H H Histochemical diagnosis of Hirschsprung s disease *Lancet II* 436 1969
7 Holschneider A M Kellner E Streibl P & Sippell W G The development of anorectal continence and its significance in the diagnosis of Hirschsprung s disease *J Pediat Surg 11* 151 1976
8 Lake B D Puri P Nixon H H & Claireaux A E Hirschsprung s disease An appraisal of histochemically demonstrated acetylcholinesterase activity in suction rectal biopsy specimens as an aid to diagnosis *Arch Pathol Lab Med 102* 244 1978
9 Lawson J O N & Nixon H H Anal canal pressures in the diagnosis of Hirschsprung s disease *J Pediatr Surg 2* 544 1967
10 Lynn H B Personal experience with rectal myectomy in the treatment of selected cases of aganglionic megacolon *Z Kinderchir 5* Suppl *98* 1968
11 Meier Ruge W Lutterbeck P M Herzog B Morger R Moser R & Scharli A Acetylcholinesterase activity in suction biopsies of the rectum in the diagnosis of Hirschsprung s disease *J Pediatr Surg 7* 11 1972
12 Nissan S Bar Maor J A & Levy E Anorectal myomectomy in the treatment of short segment Hirschsprung s disease *Ann Surg 170* 969 1969
13 Pease P W Corcery J J & Cameron A H Diagnosis of Hirschsprung s disease by punch biopsy of rectum *Arch Dis Child 51* 541 1976
14 Schnaufer L Talbert J L Haller J A Reid N C R W Tabon F & Schuster M M Differential sphincteric studies in the diagnosis of ano-rectal disorders of childhood *J Pediatr Surg 2* 538 1967
15 Shandling B & Auldist A W Punch biopsy of the rectum for the diagnosis of Hirschsprung s disease *J Pediatr Surg 7* 386 1968
16 Smith B Pre and postnatal development of the ganglion cells of the rectum and its surgical implications *J Pediatr Surg 3* 386 1968
17 Swenson O Neuhauser E B D & Picket L K New concepts of etiology diagnosis and treatment of congenital megacolon *Pediatrics 4* 201 1949
18 Swenson C Sherman J O & Fisher J H Diagnosis of congenital megacolon an analysis of 501 patients *J Pediatr Surg 8* 587 1973
19 Trigg P H Belin R Haberkorn S Long W J Nixon H H Plaschkes J Spitz L & Willital G H Experience with a cholinesterase histochemical technique for rectal suction biopsies in the diagnosis of Hirschsprung s disease *J Clin Pathol 27* 207 1974

Submitted Nov 13 1978
Accepted April 11 1979

(M K) Department of Paediatrics
Turku University Central Hospital
SF 20520 Turku
Finland

Table 3. *Clustering of features suggestive of CIA in six patients from non-CIA group*
Positivity (+) denotes findings suggestive of CIA

Patient No.	Sex	Age (years)	Neonatal history	Barium enema	Manometry	Search for ganglia	ACE	Note
1	♀	1/12	Normal	+	+	(ᵇ)	+	Congenital hypothyroidism, no symptoms
2	♂	2/12	Meteorismus	+	–	–	±	No symptoms
3	♀	3/12	Defecation difficulties	+	( )	+	–	No symptoms
4	♀	6/12	Normal	+	–	–	±	21 trisomy
5	♂	1	Defecation difficulties	–	( )	(ᵇ)	+	Uses laxatives irregularly
6	♂	8	Normal	+	–	–	±	Epidermolysis bullosa, anal fissure

For footnotes, see Table 1.

gestive of CIA. In the light of our experience these patients may be treated conservatively.

The second subgroup containing 6 infants and children with two or three diagnostic features indicating CIA, constitute a diagnostic dilemma (Table 3). The diversity of the findings may reflect the inclusion of a few cases with an ultrashort aganglionotic segment. It is therefore our present practice to stage therapy according to the clinical picture in patients with discrepant findings: if conservative treatment fails, anorectal myectomy is performed both for diagnostic and therapeutic purposes (10, 12).

The percentage of biopsies taken too superficially for proper evaluation is much higher than that reported by Campbell & Noblett (3), for example. This may reflect our wish not to perform too deep biopsies, especially in an asymptomatic neonate. Despite our carefulness we saw one serious complication in this series: a rectal perforation in a neonate occurring during suction biopsy. Significant and even lethal bleeding can complicate a punch biopsy (13, 15), though more rarely than in the case of open surgical biopsy.

In our experience the results obtained by manometry appeared to be compatible with those reported by Aaronson & Nixon (1). However, to limit the technical pitfalls in manometry, it seems to be essential to have balloon systems of different sizes at one's disposal, as also suggested by El Shafie et al. (4).

The determination of acetylcholinesterase in the rectal mucosa is probably the most significant advancement in the diagnosis of CIA. Meier-Ruge et al. (11) are credited for the introduction of ACE determinations in clinical practice. Also in this series ACE determinations proved to be a method of great value. However, its specificity was somewhat reduced by the finding of an intermediate form of ACE distribution in three cases (Table 3).

Table 4. *Distribution of symptoms and clinical findings in 60 infants and children with suspected CIA*
The features evaluated are:
signs of neonatal occlusion,
absence of ganglia in rectal wall,
ACE in rectal lamina propria,
long anal canal in barium enema, and
lack of reflex relaxation of the internal sphincter in manometry

Number of positive symptoms and findings	Number of patients	Final clinical diagnosis
5	5	CIA
4	5	CIA
3	3	non-CIA
2	3	non-CIA
1	15	non-CIA
0	29	non-CIA

Otherwise our results are in agreement with the original observations of Meier Ruge et al Series with discordant results have also been published (6 19) However in a recent appraisal of a large series the determination of ACE was found to be more reliable and consistent than any of the other methods available for the diagnosis of CIA (8)

Consequently the two latest non invasive techniques employed in the diagnosis of CIA manometry and ACE seem to be of equal value to the classic ones They are recommended for routine use at least in borderline cases

ACKNOWLEDGEMENT

This study was supported in part by grants from the Sigrid Juselius and Paulo Foundations Helsinki Finland

REFERENCES

1 Aaronson I & Nixon H H A clinical evaluation of anorectal pressure studies in the diagnosis of Hirschsprung s disease *Gut 13* 138 1972

2 Aldridge R T & Campbell P E Ganglion cell distribution in the normal rectum and anal canal A basis for the diagnosis of Hirschsprung s disease by anorectal biopsy *J Pediatr Surg 3* 475 1968

3 Campbell P E & Noblett H R Experience with rectal suction biopsy in the diagnosis of Hirschsprung s disease *J Pediatr Surg 4* 410 1969

4 El Shafie M Suzuki H Schnaufer L Haller J A & White J J A simplified method of anorectal manometry for wider clinical application *J Pediat Surg 7* 230 1972

5 Ehrenpreis T Megacolon in the newborn A clinical and roentgenological study with special regard to the pathogenesis *Acta Chir Scand 94* Suppl *112* 1946

6 Garrett J R Howard E R & Nixon H H Histochemical diagnosis of Hirschsprung s disease *Lancet II* 436 1969

7 Holschneider A M Kellner E Streibl P & Sippell W G The development of anorectal continence and its significance in the diagnosis of Hirschsprung s disease *J Pediat Surg 11* 151 1976

8 Lake B D Puri P Nixon H H & Claireaux A E Hirschsprung s disease An appraisal of histochemically demonstrated acetylcholinesterase activity in suction rectal biopsy specimens as an aid to diagnosis *Arch Pathol Lab Med 102* 244 1978

9 Lawson J O N & Nixon H H Anal canal pressures in the diagnosis of Hirschsprung s disease *J Pediatr Surg 2* 544 1967

10 Lynn H B Personal experience with rectal myectomy in the treatment of selected cases of aganglionic megacolon *Z Kinderchir 5* Suppl *98* 1968

11 Meier Ruge W Lutterbeck P M Herzog B Morger R Moser R & Scharli A Acetylcholinesterase activity in suction biopsies of the rectum in the diagnosis of Hirschsprung s disease *J Pediatr Surg 7* 11 1972

12 Nissan S Bar Maor J A & Levy E Anorectal myomectomy in the treatment of short segment Hirschsprung s disease *Ann Surg 170* 969 1969

13 Pease P W Corcery J J & Cameron A H Diagnosis of Hirschsprung s disease by punch biopsy of rectum *Arch Dis Child 51* 541 1976

14 Schnaufer L Talbert J L Haller J A Reid N C R W Tabon F & Schuster M M Differential sphincteric studies in the diagnosis of ano-rectal disorders of childhood *J Pediatr Surg 2* 538 1967

15 Shandling B & Auldist A W Punch biopsy of the rectum for the diagnosis of Hirschsprung s disease *J Pediatr Surg 7* 386 1968

16 Smith B Pre and postnatal development of the ganglion cells of the rectum and its surgical implications *J Pediatr Surg 3* 386 1968

17 Swenson O Neuhauser E B D & Picket L K New concepts of etiology diagnosis and treatment of congenital megacolon *Pediatrics 4* 201 1949

18 Swenson O Sherman J O & Fisher J H Diagnosis of congenital megacolon an analysis of 501 patients *J Pediatr Surg 8* 587 1973

19 Trigg P H Belin R Haberkorn S Long W J Nixon H H Plaschkes J Spitz L & Willital G H Experience with a cholinesterase histochemical technique for rectal suction biopsies in the diagnosis of Hirschsprung s disease *J Clin Pathol 27* 207 1974

Submitted Nov 13 1978
Accepted April 11 1979

(M K) Department of Paediatrics
Turku University Central Hospital
SF 20520 Turku
Finland

Table 3 *Clustering of features suggestive of CIA in six patients from non CIA group*
Positivity (+) denotes findings suggestive of CIA

Patient No	Patient Sex	Patient Age (years)	Neonatal history	Barium enema	Manometry	Search for ganglia	ACE	Note
1	♀	1/12	Normal	+	+	(a)	+	Congenital hypothyroidism no symptoms
2	♂	2/12	Meteorismus	+	–	–	±	No symptoms
3	♀	3/12	Defecation difficulties	+	( )	+	–	No symptoms
4	♀	6/12	Normal	+	–	–	±	21 trisomy
5	♂	1	Defecation difficulties	–	( )	(b)	+	Uses laxatives irregularly
6	♂	8	Normal	+	–	–	±	Epidermolysis bullosa anal fissure

For footnotes see Table 1

gestive of CIA In the light of our experience these patients may be treated conservatively

The second subgroup containing 6 infants and children with two or three diagnostic features indicating CIA constitute a diagnostic dilemma (Table 3) The diversity of the findings may reflect the inclusion of a few cases with an ultrashort aganglionotic segment It is therefore our present practice to stage therapy according to the clinical picture in patients with discrepant findings if conservative treatment fails anorectal myectomy is performed both for diagnostic and therapeutic purposes (10 12)

The percentage of biopsies taken too superficially for proper evaluation is much higher than that reported by Campbell & Noblett (3) for example This may reflect our wish not to perform too deep biopsies especially in an asymptomatic neonate Despite our carefulness we saw one serious complication in this series a rectal perforation in a neonate occurring during suction biopsy Significant and even lethal bleeding can complicate a punch biopsy (13 15) though more rarely than in the case of open surgical biopsy

In our experience the results obtained by manometry appeared to be compatible with those reported by Aaronson & Nixon (1) However to limit the technical pitfalls in manometry it seems to be essential to have balloon systems of different sizes at one s disposal as also suggested by El Shafie et al (4)

The determination of acetylcholinesterase in the rectal mucosa is probably the most significant advancement in the diagnosis of CIA Meier Ruge et al (11) are credited for the introduction of ACE determinations in clinical practice Also in this series ACE determinations proved to be a method of great value However its specificity was somewhat reduced by the finding of an intermediate form of ACE distribution in three cases (Table 3)

Table 4 *Distribution of symptoms and clinical findings in 60 infants and children with suspected CIA*
The features evaluated are
signs of neonatal occlusion
absence of ganglia in rectal wall
ACE in rectal lamina propria
long anal canal in barium enema and
lack of reflex relaxation of the internal sphincter in manometry

Number of positive symptoms and findings	Number of patients	Final clinical diagnosis
5	5	CIA
4	5	CIA
3	3	non-CIA
2	3	non CIA
1	15	non CIA
0	29	non-CIA

Otherwise our results are in agreement with the original observations of Meier Ruge et al Series with discordant results have also been published (6 19) However in a recent appraisal of a large series the determination of ACE was found to be more reliable and consistent than any of the other methods available for the diagnosis of CIA (8)

Consequently the two latest non invasive techniques employed in the diagnosis of CIA manometry and ACE seem to be of equal value to the classic ones They are recommended for routine use at least in borderline cases

ACKNOWLEDGEMENT

This study was supported in part by grants from the Sigrid Juselius and Paulo Foundations Helsinki Finland

REFERENCES

1 Aaronson I & Nixon H H A clinical evaluation of anorectal pressure studies in the diagnosis of Hirschsprung s disease *Gut 13* 138 197?
2 Aldridge R I & Campbell P E Ganglion cell distribution in the normal rectum and anal canal A basis for the diagnosis of Hirschsprung s disease by anorectal biopsy *J Pediatr Surg 3* 475 1968
3 Campbell P E & Noblett H R Experience with rectal suction biopsy in the diagnosis of Hirschsprung s disease *J Pediatr Su g 4* 410 1969
4 El Shafie M Suzuki H Schnaufer L Haller J A & White J J A simplified method of anorectal manometry for wider clinical application *J Pedi tr Su g 7* ?30 197
5 Ehrenpreis T Megacolon in the newborn A clinical and roentgenological study with special regard to the pathogenesis *Acta Chir Scand 94* Suppl *112* 1946
6 Garrett J R Howard E R & Nixon H H Histochemical diagnosis of Hirschsprung s disease *L ncet II* 436 1969
7 Holschneider A M Kellner E Streibl P & S ppell W G The development of anorectal continence and its significance in the diagnosis of Hirschsprung s disease *J Pedi tr Su g 11* 151 1976
8 Lake B D Puri P Nixon H H & Claireaux A E Hirschsprung s disease An appraisal of histochemically demonstrated acetylcholinesterase activity in suction rectal biopsy specimens as an aid to diagnosis *Arch Pathol Lab Med 10?* 244 1978
9 Lawson J O N & Nixon H H Anal canal pressures in the diagnosis of Hirschsprung s disease *J Pediatr Surg 2* 544 1967
10 Lynn H B Personal experience with rectal myectomy in the treatment of selected cases of aganglionic megacolon *Z Kinderchir 5* Suppl *98* 1968
11 Meier Ruge W Lutterbeck P M Herzog B Morger R Moser R & Scharli A Acetylcholinesterase activity in suction biopsies of the rectum in the diagnosis of Hirschsprung s disease *J Pediatr Surg* 7 11 1972
1? Nissan S Bar Maor J A & Levy E Anorectal myomectomy in the treatment of short segment Hirschsprung s disease *Ann Surg 170* 969 1969
13 Pease P W Corcery J J & Cameron A H Diagnosis of Hirschsprung s disease by punch biopsy of rectum *Arch Dis Child 51* 541 1976
14 Schnaufer L Talbert J L Haller J A Reid N C R W Tabon F & Schuster M M Differential sphincteric studies in the diagnosis of ano-rectal disorders of childhood *J Pediatr Surg 2* 538 1967
15 Shandling B & Auldist A W Punch biopsy of the rectum for the diagnosis of Hirschsprung s disease *J Pediatr Surg 7* 386 1968
16 Smith B Pre and postnatal development of the ganglion cells of the rectum and its surgical implications *J Pediatr Su g 3* 386 1968
17 Swenson O Neuhauser E B D & Picket L K New concepts of etiology diagnosis and treatment of congenital megacolon *Pediatrics 4* 01 1949
18 Swenson C Sherman J O & Fisher J H Diagnosis of congenital megacolon an analysis of 501 patients *J Pediatr Surg 8* 587 1973
19 Trigg P H Belin R Haberkorn S Long W J Nixon H H Plaschkes J Spitz L & Willital G H Experience with a cholinesterase histochemical technique for rectal suction biopsies in the diagnosis of Hirschsprung s disease *J Clin Pathol 27* ?07 1974

Submitted Nov 13 1978
Accepted April 11 1979

(M K) Department of Paediatrics
Turku University Central Hospital
SF 05 0 Turku
Finland

Acta Pædiatr Scand 68 899–901 1979

EFFECT OF PREGNANCY ANAEMIA ON CELLULAR GROWTH IN THE HUMAN PLACENTA

PURNIMA MARWAH P N SINGLA MEENAKSHI KRISHNA and K N AGARWAL

From the Paediatric Haematology Unit Department of Paediatrics Institute of Medical Sciences Varanasi India

ABSTRACT Marwah P Singla P N Krishna M and Agarwal K N (Paediatric Haematology Unit Department of Paediatrics Institute of Medical Sciences Varanasi India) Effect of pregnancy anaemia on cellular growth in the human placenta Acta Paediatr Scand 68 899 1979 —The effects of pregnancy anaemia on cell number and size in human placentae were investigated Total organ weight protein RNA and DNA contents were determined in 54 fresh placentae from anaemic women (haemoglobin <110 g/l) and another 32 placentae from women without anaemia (haemoglobin ≥110 g/l) The placental weight was significantly reduced in pregnancy anaemia The decrease in total placental DNA in anaemic women suggested that the reduced placental weight was due to a decrease in cell number However these placentae also showed evidence of compensatory cellular hypertrophy as indicated by increase in both weight per cell and protein per cell

KEY WORDS Pregnancy anaemia placenta RNA DNA

Several investigators have attempted to elucidate biochemical and physiologic mechanisms by which the placenta is able to support the growth and development of the foetus in maternal anaemia (3 8 9 11 12) The placenta itself is a rapidly growing organ However only few studies (1 2) have been carried out on placental growth in pregnancy anaemia and the actual cellular events occurring during this growth have not been elucidated Since cellular DNA is located almost entirely within the nucleus and is constant in amount within the diploid nucleus of any species the total quantity of DNA in a tissue or organ reflects the number of cells The ratio of total organ weight or protein content to DNA content is a measure of cell size and the RNA/DNA ratio determines the average RNA content per cell (4 13 14)

The objective of the present study was to determine the effects of pregnancy anaemia on cell division and cell growth in the human placenta utilizing the measurements of total organ weight protein RNA and DNA contents

MATERIAL AND METHODS

Fifty four fresh human placentae from anaemic women (haemoglobin <110 g/l) were studied Another 32 placentae from women with haemoglobin 110 g/l or above at the time of delivery served as controls All these women had singleton livebirths with gestation ranging from 37 to 41 weeks They had no racial cultural or environmental differences The women with preterm delivery ante partum haemorrhage toxaemia of pregnancy and blood group incompatibility were excluded from this study and so were women with systemic diseases which could have affected the placental growth

The haemoglobin was estimated by cyanmethaemoglobin method (5) on venous blood taken during the first stage of labour As soon as the placenta was delivered the umbilical cord was cut flush with the *placental surface* and the membranes were trimmed off Blood clots adhering to the placenta were removed and the subchorionic vessels were emptied of blood by gentle pressure The placenta was blotted several times with filter paper and weighed

For measurement of the placental protein RNA and DNA *contents* 1 0 g of the tissue was taken from the maternal surface of the placenta at a distance of about 1 0 cm from the cord and homogenized in 5 0 ml of ice cold distilled water for 3 min using a Potter Elvehjem homogenizer fitted with a teflon pestle Protein was determined in the homogenate by the method of Lowry et al (7) RNA and DNA were determined using the method of Schneider (10)

Table 1 *Placental weight and some biochemical characteristics in pregnancy anaemia (mean ± S D)*

Figures in parentheses indicate sample size

Group	Range of haemoglobin (g/l)	Placenta Weight (g)	Protein (g)	RNA (mg)	DNA (mg)
I	≤60 (12)	337±73	52 3±14 6	946 6±352 8	1 222 2±378 4
II	61–85 (15)	384±41	67 0±12 0	1 275 9±323 0	1 570 5±313 1
III	86–109 (27)	390±55	66 4±14 5	1 310 3±415 3	1 551 8±369 5
IV	≥110 (32)	430±46	74 3±20 6	1 681 2±577 8	1 946 8±478 2
Total observations (86)		*397±61*	*67 5±17 9*	*1 391 6±521 4*	*1 656 1±472 2*
p value					
I/IV		<0 001	<0 001	<0 001	<0 001
II/IV		<0 005	n s	<0 005	<0 005
III/IV		<0 005	n s	<0 01	<0 001

n s =not significant

RESULTS

Table 1 shows the distribution of women in four haemoglobin groups These groups were comparable in terms of gestational age parity and absence of severe morbidity during pregnancy It can be further seen that the placental weights and RNA and DNA values expressed per whole placenta were significantly low in women with various grades of anaemia (groups *I II & III*) compared to the non anaemic women (group IV) The protein values expressed for whole placenta were however significantly low only in the women with severe anaemia (haemoglobin ≤60 g/l)

Table 2 shows that the average placental weight/DNA and protein/DNA ratios were significantly high in the anaemic women while the average RNA/DNA ratio remained unaltered

DISCUSSION

The results of the present study demonstrate that the placental weight is significantly low in anaemic women indicating that placental

Table 2 *Effect of pregnancy anaemia on weight protein and RNA per cell in the placenta (mean ± S D)*

Figures in parentheses indicate sample size

Group	Range of haemoglobin (g/l)	Placenta Weight/DNA (g/g)	Protein/DNA (g/g)	RNA/DNA (mg/mg)
I	≤60 (12)	287 1±53 1	44 3±8 8	0 90±0 23
II	61–85 (15)	256 4±31 6	43 5±7 6	0 82±0 17
III	86–109 (27)	258 9±53 0	43 4±6 8	0 85±0 18
IV	≥110 (32)	231 2±48 7	38 1±6 3	0 85±0 17
Total observations (86)		*252 1±51 4*	*41 6±7 8*	*0 84±0 18*
p value				
I/IV		<0 005	<0 05	n s
II/IV		<0 05	<0 025	n s
III/IV		<0 05	<0 005	n s

n s =not significant

growth is retarded in pregnancy anaemia This is contrary to the observations of Beischer et al (2) and Agboola (1) that pregnancy anaemia is associated with a large placenta

The lower DNA content in the placentae of anaemic women in this study indicates that the decreased organ size is a consequence of a decrease in cell number Since cell division does not occur in the human placenta after 36 weeks of gestation (4 6) it can be concluded that the decreased placental DNA content is due to earlier reduction in cell division However cell size as indicated by total organ weight/DNA and protein/DNA ratios is significantly increased in these placentae suggesting cellular hypertrophy The significance of placental cellular hypertrophy evoked by pregnancy anaemia is considerable because it may indicate an adaptation to a physiological stress resulting in an improvement of placental function and foetal well being

The decrease in RNA content parallels the decrease in DNA content in the placentae of anaemic women The resulting RNA/DNA ratio does not show any change indicating that the amount of RNA per cell in the placenta remains constant in pregnancy anaemia

REFERENCES

1 Agboola A Placental changes in patients with a low haematocrit *B J Obstet Gynaecol 82* 225 1975

2 Beischer N A Sivasamboo R Vohra S Silpisornkosal S & Reid S Placental hypertrophy in severe pregnancy anaemia *J Obstet Gynaecol Br Commwlth* 77 398 1970

3 Bothwell T H Pribilla W F Mebust W & Finch C A Iron metabolism in the pregnant rabbit Iron transport across the placenta *Am J Physiol 193* 615 1958

4 Brasel J A & Winick M Maternal nutrition and placental growth Experimental studies of effects of maternal undernutrition on foetal and placental growth *Arch Dis Child 47* 479 1972

5 Crosby W H Munn J I & Furth F W Standardizing a method for clinical haemoglobinometry *US Armed Forces Med J 5* 693 1954

6 Laga E M Driscoll S G & Munro H N Comparison of placentas from two socio-economic groups II Biochemical characteristics *Pediatrics 50* 33 1972

7 Lowry O H Rosebrough N J Farr H I & Randall R J Protein measurement with Folin phenol reagent *J Biol Chem 193* 265 1951

8 McLaurin L P & Cotter J R Placental transfer of iron *Am J Obstet Gynecol 98* 931 1967

9 Nhonoli A M Kihama F E & Ramji B D The relation between maternal and cord serum iron levels and its effect on foetal growth in iron deficient mothers without malarial infection *Br J Obstet Gynaecol 82* 467 1975

10 Schneider W E Determination of nucleic acids in tissues by pentose analysis *In* S P Colowick & N O Kaplan (eds) *Methods in enzymology* Academic Press Inc New York 1957 *vol III p 680*

11 Singla P N Chand S Khanna S & Agarwal K N Effect of maternal anaemia on the placenta and the newborn infant *Acta Paediat Scand 67* 645 1978

12 Singla P N Chand S & Agarwal K N Cord serum and placental tissue iron status in maternal hypoferremia *Am J Clin Nutr* (in press)

13 Winick M Coscia A & Noble A Cellular growth in human placenta I Normal placental growth *Pediatrics 39* 248 1967

14 Zeman F J & Stanbrough E C Effect of maternal protein deficiency on cellular development in the fetal rat *J Nutr* 99 274 1969

Submitted April 6 1979
Accepted May 29 1979

(P N S) Paediatric Haematology Unit
Department of Paediatrics
Institute of Medical Sciences
Banaras Hindu University
Varanasi 221005
India

Acta Pædiatr Scand 68 903–909 1979

INCREASED OSMOTIC FRAGILITY OF ERYTHROCYTES IN CHRONICALLY JAUNDICED RATS AFTER PHOTOTHERAPY

JULIO O CUKIER APOLO C MAGLALANG and GERARD B ODELL

From the Department of Pediatrics Johns Hopkins University School of Medicine Baltimore Maryland and the Department of Pediatrics University of Wisconsin School of Medicine Madison Wisconsin USA

ABSTRACT Cukier J O Maglalang A C and Odell G B (Department of Pediatrics Johns Hopkins University School of Medicine Baltimore Maryland and the Department of Pediatrics University of Wisconsin School of Medicine Madison Wisconsin) Increased osmotic fragility of erythrocytes in chronically jaundiced rats after phototherapy Acta Paediatr Scand 68 903 1979.—Littermate homozygous (jj) and heterozygous (Jj) Gunn rats were irradiated with blue fluorescent light for 18 hours continuously The incident irradiance was 1 5 mWatts/cm in the 470–480 nm band pass The influence of the irradiance on circulating erythrocytes was studied by testing their osmotic fragility before and after the irradiance The non jaundiced Jj animals did not exhibit any increase in the osmotic fragility of their erythrocytes The osmotic fragility of the erythrocytes from jaundiced jj animals was the same as the Jj animals prior irradiance However the fragility of the erythrocytes from the jj animals was significantly increased after the 18 hours of irradiance The results indicate that the photodynamic action of bilirubin may be present in vivo

KEY WORDS Bilirubin phototherapy photosensitized hemolysis osmotic fragility Gunn rat

In a previous report it was suggested that phototherapy for neonatal hyperbilirubinemia might actually increase the formation of bilirubin because of the capacity of bilirubin to produce light induced hemolysis (22) in vitro and the failure of some infants treated by phototherapy to show significant reductions in their concentrations of serum bilirubin (26)

Since Saeki s original observations (29) it has been recognized that bilirubin is a photosensitizing agent and can produce hemolysis when within the erythrocyte in the presence of oxygen and light (22) i e it is a photodynamic agent (1) The basis for its photodynamic action has been verified by its ability to generate singlet oxygen (20) and thus resembles many photosensitizing hemolytic compounds (21 30) As with other photolytic compounds in order for bilirubin to induce photosensitized hemolysis it must be within the red-cell membrane (22 24)

Photosensitized hemolysis has been historically characterized into immediate hemolysis which is the hemolysis that occurs during the light exposure and delayed or after light hemolysis which reflects the continuing injury to the red cell even after the cessation of irradiance (1)

Associated with the hemolysis induced in vitro by bilirubin are alterations in the enzymatic activities of erythrocyte membranes These include reduction in Mg^{2+} ATPase and Na^+ K^+ ATPase (9 16 22) acetylcholinesterase and glyceraldehyde 3 phosphate dehydrogenase (9 10) Chemical alterations of photosensitized erythrocytes secondary to singlet oxygen ($^1O_2$) formation have also been described with other porphyrin pigments and include peroxidation of unsaturated membrane lipids (12) and cholesterol (8) as well as polypeptide aggregation (11) Structural changes consist of the formation of spherocytes (22)

Table 1 *Comparative responses of homozygous and heterozygous Gunn rats to 18 hrs of continuous phototherapy*

Animal	Weight (g)	Hematocrit (%)	Hemoglobin (g/dl)	Total plasma protein (g/dl)	Plasma bilirubin (mg/dl)
jj_1	239	50[a]	15.3	5.5	4.7
	NW	47	16.0	5.8	3.0
jj_2	273	50	14.8	6.0	6.8
	243	49.5	14.0	6.0	6.8
jj_3	353	47	13.3	6.0	10.0
	345	50	14.7	6.0	7.5
jj_4	347	49.5	16.4	6.2	7.8
	337	46.5	15.2	6.0	7.5
Jj_1	251	47.5	13.6	7.0	
	NW	44	12.4	7.0	
Jj_2	563	51	13.7	6.5	
	535	51	16.2	6.5	
Jj_3	400	50.5	16.2	6.0	
	379	51.5	16.0	6.0	
Jj_4	382	49.5	16.2	6.0	
	373	48.0	15.7	6.2	

[illegible] Not weighed [a] Pre phototherapy [b] Post phototherapy

The translation of the above in vitro actions of bilirubin to in vivo circumstances must be done with caution for the action spectrum of bilirubin in vivo is not known and the wavelengths of light responsible for the reduction clinically in serum concentrations of bilirubin may not be the same as the wavelengths responsible for its photosensitizing hemolytic effects The studies of Vogl (32) indicated that the radiant energy from broad spectrum fluorescent lights that are responsible for the reduction of skin bilirubin concentrations apparently penetrate no more than the first 2 mm of the skin However in vivo for light to produce a photosensitized hemolysis the incident radiant energy must not only penetrate the epidermis but also extravascular tissues of the dermis and the vascular endothelium before it can reach circulating erythrocytes Furthermore one might anticipate only those erythrocytes within the very periphery of the capillary lumen would be subject to the photosensitized hemolytic action of bilirubin for the hemoglobin within erythrocytes strongly absorb the radiant energy between 400–500 nm which are thought to be the wavelengths of light essential for the photodynamic effects of bilirubin (31) Thus despite the well documented photolytic action of bilirubin on isolated erythrocytes in vivo circumstances may militate against any photodynamic hemolytic action in clinical circumstances

In order to test the influence of the radiant energy of fluorescent lights used clinically for phototherapy on circulating erythrocytes in vivo we studied the integrity of erythrocytes from homozygous jaundiced (jj) and littermate heterozygous (Jj) Gunn strain of rats subjected to 18 hours of continuous phototherapy with blue fluorescent lights After completion of these studies contradictory reports have been published indicating that fluorescent irradiation of jaundiced Gunn rats does and does not influence red cell survival (14 28)

MATERIALS AND METHODS

Four pair of non icteric heterozygous (Jj) and jaundiced homozygous (jj) littermates were selected and when the animals weighed 200 g they were splenectomized under ether anaesthesia Four to six weeks after splenectomy the Jj and jj littermates were again anaesthetized with ether and their dorsal and lateral skin removed by an electric clipper While anaesthetized 0 8 ml of blood was collected from the tail into heparinized ([illegible] ml capacity) glass tubes before and after the 18 hours of fluorescent

Table 2 *The immediate and delayed osmotic fragility of erythrocytes from Gunn rats before and after 18 hours of continuous phototherapy*

Osmolality (mOsm/kg)	Pre photo Rx		Post photo Rx	
	jj (*n*=4)	Jj (*n*=4)	jj (*n*=4)	Jj (*n*=4)
Immediate hemolysis (%)				
? 0	0 4± 0 17	0 ? ±0 10	0 21± 0 11	0 16±0 04
07	0 ?7± 0 16	0 1±0 10	0 9± 0 09	0 30±0 14
135	0 89± 0 8	0 55±0 ?3	1 9± 0 81	0 45±0 93
118	15 41± 9 50	1 19±5 44	14 80±10 83	13 73±6 0
10	43 77± 8 91	5? 90±9 56	45 69±13 19	4 67±9 ??
90	66 96±13 7	74 3 ±7 09	71 75±19 07	71 71±9 67
57	8? 89±13	87 65±8 4	81 31±13 76	86 1?±6 83
0	83 ? ±14 61	93 01±8 08	84 05±1 30	86 08±3 98
Delayed hemolysis (%)				
70	0 46±0 ?7	0 48±0 ?7	0 36±0 18	0 3?±0 ??
07	0 49±0 18	0 5?±0 17	0 78±0 69[a]	0 ?7±0 08
135	1 ??±0 56	1 ?4±0 40	? 98±3 01	0 86±0 15

jj vs jj pre and post photo Rx $p<0.05$
[a] jj vs Jj post photo Rx $p<0.01$
jj vs Jj post photo Rx $p<0.001$

light irradiance The tail of the anaesthetized animal was warmed in hot tap water and the tip incised with a scalpel blade The blood was collected by gentle milking of the incised tail permitting free flow into heparinized glass tubes After the sample collection hemostasis was accomplished by coagulation by application of a pre heated metal spatula to the incised tail

For the irradiance exposure the animals were placed in a plastic cage containing maple wood shavings and food pellets The top of the cage was covered by a stainless steel slotted grid which supported a water bottle to which the animals had free access during the experiment The fluorescent irradiance source was from a bank of 10 fluorescent lights (SE F?D T1?/B) posi tioned 60 cm above the cage The incident radiant energy at the top of the cage was 1 5 mWatts/cm be tween the 4 0–480 nm band pass The light energy was measured with a bread board model selenium irra diance meter equipped with a neutral density and Wrat ten (no 98) filters Calibration of the light meter was done at the Applied Physics Laboratory of the Johns Hopkins University and the National Bureau of Stand ards (14) The duration of irradiance was 18 hours con tinuously

The pre and post phototherapy blood samples were handled identically The immediate and de layed light induced effects on the erythrocytes was quantitated by the changes in osmotic fragility after the method of Cartwright (4)

To 0 5 ml of phosphate buffered solutions of saline (pH 7 4) 50 μl of whole blood was pipetted into dupli cate sets of tubes The osmolalities of the solutions ranged between 0 and ? 0 mOsm/kg One set was allowed to stand immediately after mixing for 30 min at 25°C The other set which consisted of blood samples added to buffered solutions of 0 07 and 135 mOsm/kg , were covered and refrigerated at 4 C for 24 hours in the dark

After 30 min of incubation the samples immediately studied were centrifuged at 3000×*g* for 10 min From the supernatant appropriate aliquots were taken for deter mination of hemoglobin by the benzidine technique of Crosby (6) The loss of erythrocyte hemoglobin was cal culated as percent of total hemoglobin from the super natant of a blood sample that had been completely hemolyzed by distilled water Whole blood rather than washed cells were used because in preliminary trials it was found that rat erythrocytes hemolyze while being washed in the absence of albumin

The second set of samples which had been refrige rated ?4 hours were brought to 25 C incubated 30 min and then analyzed as described above The latter samples represented the delayed or after light in jury to the erythrocyte population

The osmolality of the phosphate buffered solutions of saline was measured in a vapor pressure osmometer (Microlab model no 301) at 37°C

From the residual samples of heparinized blood the whole blood hematocrits were determined in duplicate by filling 70 μl capillary hematocrit tubes and subse quently centrifuging them The remaining blood was transferred to Natelson capillary tubes and centrifuged at 3000×*g* for 10 min The supernatant plasmas were analyzed for total protein concentration by a biuret technique (13) and in the instances of the jj jaundiced animals total bilirubin concentrations were measured colorimetrically after chemical conversion to the azodi pyrrolic sulfanilate derivatives (17)

The bilirubin content of the erythrocytes in jaundiced rats was measured in 5 animals of comparable age to the study animals Under ether anaesthesia blood was aspi rated from the abdominal aorta into a heparinized

Table 1 *Comparative responses of homozygous and heterozygous Gunn rats to 18 hrs of continuous phototherapy*

Animal	Weight (g)	Hematocrit (%)	Hemoglobin (g/dl)	Total plasma protein (g/dl)	Plasma bilirubin (mg/dl)
jj1	239	50[b]	15.3	5.5	4.7
	NW[a]	47	16.0	5.8	3.0
jj2	273	50	14.8	6.0	6.8
	243	49.5	14.0	6.0	6.8
jj3	353	47	13.3	6.0	10.0
	345	50	14.7	6.0	7.5
jj4	347	49.5	16.4	6.2	7.8
	337	46.5	15.2	6.0	7.5
Jj1	251	47.5	13.6	7.0	
	NW	44	12.4	7.0	
Jj2	563	51	13.7	6.5	
	535	51	16.2	6.5	
Jj3	400	50.5	16.2	6.0	
	379	51.5	16.0	6.0	
Jj4	382	49.5	16.2	6.0	
	373	48.0	15.7	6.2	

[a] Not weighed [b] Pre phototherapy [c] Post phototherapy

The translation of the above in vitro actions of bilirubin to in vivo circumstances must be done with caution for the action spectrum of bilirubin in vivo is not known and the wavelengths of light responsible for the reduction clinically in serum concentrations of bilirubin may not be the same as the wavelengths responsible for its photosensitizing hemolytic effects The studies of Vogl (32) indicated that the radiant energy from broad spectrum fluorescent lights that are responsible for the reduction of skin bilirubin concentrations apparently penetrate no more than the first 2 mm of the skin However in vivo for light to produce a photosensitized hemolysis the incident radiant energy must not only penetrate the epidermis but also extravascular tissues of the dermis and the vascular endothelium before it can reach circulating erythrocytes Furthermore one might anticipate only those erythrocytes within the very periphery of the capillary lumen would be subject to the photosensitized hemolytic action of bilirubin for the hemoglobin within erythrocytes strongly absorb the radiant energy between 400–500 nm which are thought to be the wavelengths of light essential for the photodynamic effects of bilirubin (31) Thus despite the well documented photolytic action of bilirubin on isolated erythrocytes in vivo circumstances may militate against any photodynamic hemolytic action in clinical circumstances

In order to test the influence of the radiant energy of fluorescent lights used clinically for phototherapy on circulating erythrocytes in vivo we studied the integrity of erythrocytes from homozygous jaundiced (jj) and littermate heterozygous (Jj) Gunn strain of rats subjected to 18 hours of continuous phototherapy with blue fluorescent lights After completion of these studies contradictory reports have been published indicating that fluorescent irradiation of jaundiced Gunn rats does and does not influence red cell survival (14 28)

MATERIALS AND METHODS

Four pair of non icteric heterozygous (Jj) and jaundiced homozygous (jj) littermates were selected and when the animals weighed 200 g they were splenectomized under ether anaesthesia Four to six weeks after splenectomy the Jj and jj littermates were again anaesthetized with ether and their dorsal and lateral skin removed by an electric clipper While anaesthetized 0.8 ml of blood was collected from the tail into heparinized (2 ml capacity) glass tubes before and after the 18 hou[illegible] fluorescent

membrane and a secondary colloid osmotic swelling of the erythrocyte due to its contained hemoglobin (5) The membrane pump(s) can no longer maintain the concentration gradient between intra and extra erythrocyte sodium The oncotic effect of hemoglobin increases the erythrocyte volume and spherocytosis occurs which precedes the actual hemolysis

The results in littermate Gunn rats demonstrated a difference in the osmotic fragility of erythrocytes from jj and Jj animals after 18 hours of irradiance The increased susceptibility to osmotic challenge of the erythrocytes from the jj animals only indicate that the irradiance created a light induced injury unique to the jaundiced animals Since the osmotic fragility was not increased when measured immediately spherocytes were apparently not present in vivo The spherocytosis occurred during in vitro storage of the light damaged cells

Previous in vitro studies have demonstrated that if bilirubin is in the erythrocyte fluorescent irradiance can induce hemolysis in the presence of oxygen (22) In the current study it was possible to demonstrate that the circulating erythrocytes in jj animals contained an average of 0 9 mg bilirubin/100 ml of packed cells Confidence in the absolute value is subject to some reservation because its determination quantitatively depended on the small arithmetic difference between two larger values of plasma bilirubin concentrations The differences would have greater significance if the cells could have been washed free of plasma before resuspension in albumin as done by Bratlid (2) with human erythrocytes but unfortunately the rat erythrocytes hemolyzed in the absence of albumin The large amount of hemoglobin present in the supernatant fluid of so washed erythrocytes rendered the determination of the small concentrations of bilirubin even less reliable

Other investigations have also demonstrated alterations in circulating blood elements including thrombocytopenia (18) and reduction of erythrocyte ATPase (16) and acetyl salicylic acid esterase (33) associated with fluorescent irradiation in vivo of jaundiced infants Such observations indicate that some of the incident irradiance associated with phototherapy may indeed penetrate into the capillary circulation

The occurrence of increased production of bilirubin associated with phototherapy can be surmised from the earlier studies of Ostrow (25) The latter investigations using radiolabelled ^{14}C bilirubin in jaundiced rats demonstrated an 8–10 fold increase in biliary excretion of the radiolabel during fluorescent irradiance mostly as unconjugated bilirubin These observations have been confirmed (19) An accelerated decay in the specific activity of the serum bilirubin (dpm/mg bilirubin) was also noted However the serum bilirubin concentrations in some of the animals did not decrease One probable explanation is that simultaneous to the increased excretion of bilirubin was an accelerated appearance of unlabelled bilirubin into the circulation This latter unlabelled bilirubin was derived from accelerated heme protein catabolism The present studies suggest one source of this unlabelled bilirubin was from the catabolism of hemoglobin from photosensitized erythrocytes The increased reticulocytosis in jaundiced rats exposed to fluorescent irradiation (28) is also consistent with such an hypothesis Thus the failure of animals jj and jj₄ in the present report to exhibit significant reductions in their serum bilirubin concentrations after 18 hours of irradiance may simply reflect an acceleration in the formation of bilirubin that equalled its excretion rather than a failure of phototherapy to induce accelerated hepatic excretion of bilirubin

An important difference between the experiments of Ostrow (25) and McDonagh (19) and the present study was that the common bile duct was not cannulated The enterohepatic circulation was intact in the

syringe From the well mixed blood sample 0 5 ml was pipetted into two 2 ml glass tubes From the first tube the whole blood hematocrit was measured in duplicate in 70 μl capillary tubes

To the second tube 5 μl of 3% bovine albumin in isotonic saline was added and the samples equilibrated 30 min at 25 C The sample was again thoroughly mixed by inversion and the hematocrit measured in duplicate to correct for changes in the packed cell and plasma volumes induced by the oncotic effect of the added albumin on red cell water

The remainder of the blood in the tubes was then centrifuged and the bilirubin concentration of the plasma measured spectrophotometrically The plasma was diluted in 0 1 M phosphate buffer (pH 7 4) and the difference in absorbance of the samples at 450 and 540 nm was measured The difference in absorbance was divided by the extinction coefficient of bilirubin $\left(\frac{\varepsilon mg/100\ ml}{\Delta\lambda\ nm} = 0\ 79\right)$ (23) Any increase in the plasma bilirubin from the blood samples to which albumin had been added was interpreted as derived from the erythrocytes

Comparison of the mean results and standard deviations of the osmotic fragilities between pre and post phototherapy samples within jj and Jj animals and between them was statistically analyzed by Fischer s exact test and by variance ratio (3 7)

RESULTS

Table 1 records the measured values of body weight blood hematocrits plasma protein and bilirubin concentrations of the animals before and after 18 hours of continuous fluorescent irradiance

All of the animals consistently lost weight but the blood hematocrits and plasma protein concentrations were not significantly changed The reductions in plasma concentrations of bilirubin in the irradiated jj animals were variable in that jj_1 and jj_3 exhibited reductions whereas the values in animals jj and jj_4 before and after 18 hours of irradiance were not significantly different

Table 2 records the immediate and delayed osmotic fragilities of the erythrocyte populations of the animals before and after irradiance The data indicate 18 hours of irradiance by the fluorescent lamps had no influence on the immediate or delayed osmotic fragility of the erythrocyte populations in the Jj animals when exposed to progressively hypotonic solutions By contrast the osmotic fragilities delayed hemolysis were significantly greater in the blood samples from the jaundiced homozygous (jj) animals after the fluorescent irradiance When statistically analyzed comparison of the variances (F test) of the delayed hemolysis in the jj animals after the irradiation demonstrated a significantly greater population of erythrocytes which were more susceptible to osmotically induced hemolysis than before the 18 hours of irradiance Analysis of the variance in hemolysis of erythrocytes from the jj animals compared to the values of their non jaundiced littermates showed an even greater difference in osmotic fragility Of significance both biologically and statistically the jj animals exhibited increased erythrocyte fragility after 18 hours of phototherapy whereas none of their non jaundiced littermates Jj showed an increased fragility Indeed the mean values and standard deviations of the delayed fragilities in the Jj animals were smaller after 18 hours of fluorescent irradiation Tabulation of the results in a 2×2 table indicated that all of the jj animals exhibited increased osmotic fragility and none of their heterozygous littermates did so By application of Fischer s exact test these results have a combined two tailed probability value <0 029

The mean bilirubin concentration of erythrocytes in the comparable aged jj animals was 0 914 mg/100 ml of packed cells with a range of 0 1–1 9 which represented 10% of the mean plasma concentration of bilirubin in the 5 animals studied

DISCUSSION

The animals were splenectomized before the irradiance studies in order to retard the rate of removal of light damaged cells from the circulation and enhance the possibility for their detection by an increased osmotic fragility

Photosensitized hemolysis associated with bilirubin as with other photosensitizing hemolytic agents is thought to result from the photon induced injury to the erythrocyte

membrane and a secondary colloid osmotic swelling of the erythrocyte due to its contained hemoglobin (5) The membrane pump(s) can no longer maintain the concentration gradient between intra and extra erythrocyte sodium The oncotic effect of hemoglobin increases the erythrocyte volume and spherocytosis occurs which precedes the actual hemolysis

The results in litterm ite Gunn rats demonstrated a difference in the osmotic fragility of erythrocytes from jj and Jj animals after 18 hours of irradiance The increased susceptibility to osmotic challenge of the erythrocytes from the jj animals only indicate that the irradiance created a light induced injury unique to the jaundiced animals Since the osmotic fragility was not increased when *measured* immediately spherocytes were apparently not present in vivo The spherocytosis occurred during in vitro storage of the light damaged cells

Previous in vitro studies have demonstrated that if bilirubin is in the erythrocyte fluorescent irradiance can induce hemolysis in the presence of oxygen (22) In the current study it was possible to demonstrate that the circulating erythrocytes in jj animals contained an average of 0 9 mg bilirubin/100 ml of packed cells Confidence in the absolute value is subject to some reservation because its determination quantitatively depended on the small arithmetic difference between two larger values of plasma bilirubin concentrations The differences would have greater significance if the cells could have been washed free of plasma before resuspension in albumin as done by Brathd (2) with human erythrocytes but unfortunately the rat erythrocytes hemolyzed in the absence of albumin The large amount of hemoglobin present in the supernatant fluid of so washed erythrocytes rendered *the* determination of the small concentrations of bilirubin even less reliable

Other investigations have also demonstrated alterations in circulating blood elements including thrombocytopenia (18) and reduction of erythrocyte ATPase (16) and acetyl salicylic acid esterase (33) associated with fluorescent irradiation in vivo of jaundiced infants Such observations indicate that some of the incident irradiance associated with phototherapy may indeed penetrate into the capillary circulation

The occurrence of increased production of bilirubin associated with phototherapy can be surmised from the earlier studies of Ostrow (25) The latter investigations using radiolabelled ^{14}C bilirubin in jaundiced rats demonstrated an 8–10 fold increase in biliary excretion of the radiolabel during fluorescent irradiance mostly as unconjugated bilirubin These observations have been confirmed (19) An accelerated decay in the specific activity of the serum bilirubin (dpm/mg bilirubin) was also noted However the serum bilirubin concentrations in some of the *animals* did not decrease One probable explanation is that simultaneous to the increased excretion of bilirubin was an accelerated appearance of unlabelled bilirubin into *the circulation* This latter unlabelled bilirubin was derived from accelerated heme protein catabolism The present studies suggest one source of this unlabelled bilirubin was *from* the catabolism of hemoglobin from photosensitized erythrocytes The increased reticulocytosis in jaundiced rats exposed to fluorescent irradiation (28) is also consistent with such an hypothesis Thus the failure of *animals* jj_2 and jj_4 in the present report to exhibit significant reductions in their serum bilirubin concentrations after 18 hours of irradiance may simply reflect an acceleration in the formation of bilirubin that equalled its excretion rather than a failure of phototherapy to induce accelerated hepatic excretion of bilirubin

An important difference between the experiments of Ostrow (25) and McDonagh (19) and the present study was that the common bile duct was not cannulated The enterohepatic circulation was intact in the

animals of the current experiments The latter circumstance would enhance the possibility of greater intestinal absorption of unconjugated bilirubin within the intestinal lumen consequent to greater biliary excretion associated with the fluorescent irradiance (15 27) Thus even though there may have been greater hepatic excretion of bilirubin during phototherapy the opportunity for its enterohepatic circulation was also increased

In view of the well documented photosensitizing hemolytic effects of bilirubin in vitro on erythrocytes the present in vivo studies in jaundiced Gunn rats do suggest that photosensitized hemolysis may occur in infants who are treated by phototherapy Whether a photosensitized hemolysis occurs depends in large measure on whether sufficient bilirubin concentrations are within the erythrocyte s membrane Its relative rarity would indicate little erythrocyte bilirubin is present clinically at the time phototherapy is initiated

ACKNOWLEDGEMENTS

These studies have been supported by Grants HD 10932 and AM 21668 of the United States Public Health Service and reported in part at the 4th Annual Meeting of the American Society for Phototherapy February 17 1978 Denver Colorado USA

REFERENCES

1 Blum H F General photochemical aspects of photodynamic action In *Photodynamic action and diseases caused by light* American Chemical Society Monograph Series Reinhold Publishing Co New York 1941 p 47
2 Bratlid D Reserve albumin binding capacity salicylate saturation index and red cell binding of bilirubin in neonatal jaundice *Arch Dis Child 48* 393 1973
3 Brownlee K A *Industrial experimentation* Chemical Publishing Co Brooklyn NY 1953 4th ed p 34
4 Cartwright G E *Diagnostic laboratory hematology* Grune and Stratton New York 1968 4th ed p 290
5 Cook J S The quantitative interrelationships between ion fluxes cell swelling and radiation dose in ultraviolet hemolysis *J Gen Physiol 48* 719 1965
6 Crosby W H & Furth F W A modification of the benzidine method for measurement of hemoglobin in plasma and urine *Blood 11* 380 1965
7 Dixon W J & Massey F J Jr *Introduction to statistical analysis* McGraw Hill New York 1969 3rd ed p 243
8 Doleiden F H Fahrenholtz S R Lamola A A & Trozzolo A M Reactivity of cholesterol and some fatty acids toward singlet oxygen *Photochem Photobiol 20* 519 1974
9 Girotti A W Bilirubin sensitized photoinactivation of enzymes in the isolated membrane of the human erythrocyte *Photochem Photobiol 24* 525 1976
10 Girotti A W Glyceraldehyde 3 phosphate dehydrogenase in the isolated human erythrocyte membrane selective displacement by bilirubin *Archs Biochem Biophys 173* 210 1976
11 Girotti A W Photodynamic action of protoporphyrin IX on human erythrocytes cross linking of membrane proteins *Biochem Biophys Res Comm 72* 1367 1976
12 Goldstein B D & Harber L C Erythropoietic photoporphyria lipid peroxidation and red cell membrane damage associated with photohemolysis *J Clin Invest 51* 892 1972
13 Gornall A G Bardawill C J & David M M Determination of serum proteins by means of the biuret reaction *J Biol Chem 177* 751 1949
14 Howe R B Hadland C R & Engel R R Effect of phototherapy on serum bilirubin levels and red blood cell survival in congenitally jaundiced Gunn rats *J Lab Clin Med 92* 221 1978
15 Lester R & Schmid R Intestinal absorption of bile pigments I The enterohepatic circulation of bilirubin in the rat *J Clin Invest 42* 736 1963
16 John E Complications of phototherapy in neonatal hyperbilirubinemia *Aust Paediat J 11* 53 1975
17 Malloy H T & Evelyn K A The determination of bilirubin with the photoelectric colorimeter *J Biol Chem 119* 481 1937
18 Maurer H M Fratkin M McWilliams N B Kirkpatrick B Draper D Higgins J C & Hunter C R Effects of phototherapy on platelet counts in low birthweight infants and on platelet production and life span in rabbits *Pediatrics 57* 506 1976
19 McDonagh A F The photochemistry and photometabolism of bilirubin *In* G B Odell R Schafer & A Simopoulos (eds) *Phototherapy in the Newborn An Overview* Natl Acad Sciences Washington D C 1974 p 56
20 McDonagh A F The role of singlet oxygen in bilirubin photooxidation *Biochem Biophys Res Comm 44* 1306 1971
21 Nilsson R Seanbeck G & Wennersten G Primary mechanisms of erythrocyte photolysis induced in biological sensitizers and phototoxic drugs *Photochem Photobiol 22* 183 1975
22 Odell G B Brown R S & Kopelman A E The photodynamic action of bilirubin on erythrocytes *J Pediatr 81* 473 1972
23 Odell G B Cukier J O Ostrea E M Jr Maglalang A C & Poland R L The influence of fatty

acids on the binding of bilirubin to albumin J Lab Clin Med 89 795 1977
24 Ostrea E M Jr & Odell G B Photosensitized shift in the O dissociation curve of fetal blood Acta Paediatr Scand 63 341 1974
25 Ostrow J D Photocatabolism of labeled bilirubin in the congenitally jaundiced (Gunn) rat J Clin Invest 50 707 1971
26 Patel D A Pildes R S & Behrman R E Failure phototherapy to reduce serum bilirubin in newborn infants J Pediatr 77 1048 1970
27 Poland R L & Odell G B Physiologic jaundice the enterohepatic circulation of bilirubin N Engl J Med 284 1 1971
28 Robinson S H & Schunior A Hemolytic anemia induced by light therapy in jaundiced rats Proc Soc Biol Med 158 81 1978
29 Saeki K Studies in the photodynamic haemolytic action of bilirubin 1 Presence or absence of the photodynamic haemolytic action an inquiry into the factor concerned in it the after effects of the action and presence or absence of photodynamic haemolytic action biliverdin Jap J Gastroenterol 4 153 193
30 Spikes J D Photodynamic action In A C Giese (ed) Photophysiology Academic Press New York 1968 vol 3 p 33
31 Van Assendelft O W Spectrophotometry of haemoglobin derivatives Thomas Springfield Ill 1970 p 55
32 Vogl T P Phototherapy of neonatal hyperbilirubinemia bilirubin in unexposed areas of the skin J Pediatr 85 707 1974
33 Windorfer A Jr Faxelius G & Boreus L O Studies on phototherapy in newborn infants Influence on protein binding of bilirubin and salicylate and on activity of acetyl salicylic acid esterase Acta Paediatr Scand 64 293 1975
34 Final report of the committee on Phototherapy in the Newborn Natl Acad Sciences Washington D C 1974

Submitted March 30 1979
Accepted May 15 1979

(G B O) Department of Pediatrics
University of Wisconsin Clinical Science Center
600 Highland Avenue H4/4 ?
Madison Wisconsin 5379
USA

animals of the current experiments The latter circumstance would enhance the possibility of greater intestinal absorption of unconjugated bilirubin within the intestinal lumen consequent to greater biliary excretion associated with the fluorescent irradiance (15 27) Thus even though there may have been greater hepatic excretion of bilirubin during phototherapy the opportunity for its enterohepatic circulation was also increased

In view of the well documented photosensitizing hemolytic effects of bilirubin in vitro on erythrocytes the present in vivo studies in jaundiced Gunn rats do suggest that photosensitized hemolysis may occur in infants who are treated by phototherapy Whether a photosensitized hemolysis occurs depends in large measure on whether sufficient bilirubin concentrations are within the erythrocyte s membrane Its relative rarity would indicate little erythrocyte bilirubin is present clinically at the time phototherapy is initiated

ACKNOWLEDGEMENTS

These studies have been supported by Grants HD 10932 and AM 21668 of the United States Public Health Service and reported in part at the 4th Annual Meeting of the American Society for Phototherapy February 17 1978 Denver Colorado USA

REFERENCES

1 Blum H F General photochemical aspects of photodynamic action In *Photodynamic action and diseases caused by light* American Chemical Society Monograph Series Reinhold Publishing Co New York 1941 p 47

2 Bratlid D Reserve albumin binding capacity salicylate saturation index and red cell binding of bilirubin in neonatal jaundice *Arch Dis Child 48* 393 1973

3 Brownlee K A *Industrial experimentation* Chemical Publishing Co Brooklyn NY 1953 4th ed p 34

4 Cartwright G E *Diagnostic laboratory hematology* Grune and Stratton New York 1968 4th ed p 290

5 Cook J S The quantitative interrelationships between ion fluxes cell swelling and radiation dose in ultraviolet hemolysis *J Gen Physiol 48* 719 1965

6 Crosby W H & Furth F W A modification of the benzidine method for measurement of hemoglobin in plasma and urine *Blood 11* 380 1965

7 Dixon W J & Massey F J Jr *Introduction to statistical analysis* McGraw Hill New York 1969 3rd ed p 243

8 Doleiden F H Fahrenholtz S R Lamola A A & Trozzolo A M Reactivity of cholesterol and some fatty acids toward singlet oxygen *Photochem Photobiol 20* 519 1974

9 Girotti A W Bilirubin sensitized photoinactivation of enzymes in the isolated membrane of the human erythrocyte *Photochem Photobiol 24* 525 1976

10 Girotti A W Glyceraldehyde 3 phosphate dehydrogenase in the isolated human erythrocyte membrane selective displacement by bilirubin *Archs Biochem Biophys 173* 210 1976

11 Girotti A W Photodynamic action of protoporphyrin IX on human erythrocytes cross linking of membrane proteins *Biochem Biophys Res Comm 72* 1367 1976

12 Goldstein B D & Harber L C Erythropoietic photoporphyria lipid peroxidation and red cell membrane damage associated with photohemolysis *J Clin Invest 51* 892 1972

13 Gornall A G Bardawill C J & David M M Determination of serum proteins by means of the biuret reaction *J Biol Chem 177* 751 1949

14 Howe R B Hadland C R & Engel R R Effect of phototherapy on serum bilirubin levels and red blood cell survival in congenitally jaundiced Gunn rats *J Lab Clin Med 92* 221 1978

15 Lester R & Schmid R Intestinal absorption of bile pigments I The enterohepatic circulation of bilirubin in the rat *J Clin Invest 42* 736 1963

16 John E Complications of phototherapy in neonatal hyperbilirubinemia *Aust Paediat J 11* 53 1975

17 Malloy H T & Evelyn K A The determination of bilirubin with the photoelectric colorimeter *J Biol Chem 119* 481 1937

18 Maurer H M Fratkin M McWilliams N B Kirkpatrick B Draper D Higgins J C & Hunter C R Effects of phototherapy on platelet counts in low birthweight infants and on platelet production and life span in rabbits *Pediatrics 57* 506 1976

19 McDonagh A F The photochemistry and photometabolism of bilirubin *In* G B Odell R Schafer & A Simopoulos (eds) *Phototherapy in the Newborn An Overview* Natl Acad Sciences Washington D C 1974 p 56

20 McDonagh A F The role of singlet oxygen in bilirubin photooxidation *Biochem Biophys Res Comm 44* 1306 1971

21 Nilsson R Swanbeck G & Wennersten G Primary mechanisms of erythrocyte photolysis induced in biological sensitizers and phototoxic drugs *Photochem Photobiol 22* 183 1975

22 Odell G B Brown R S & Kopelman A E The photodynamic action of bilirubin on erythrocytes *J Pediatr 81* 473 1972

23 Odell G B Cukier J O Ostrea E M Jr Maglalang A C & Poland R L The influence of fatty

LETTER TO THE EDITOR

BENIGN PAROXYSMAL TORTICOLLIS IN INFANCY

Sir

I read with great interest the paper on Benign paroxysmal Torticollis in Infancy by Sanner & Bergstrom (4)

I completely agree that most of the differential diagnoses mentioned by the authors (posterior fossa tumor cervical dislocation epilepsy Sandifer s syndrome etc) lack—among *other symptoms—the regular periodicity of* paroxysmal torticollis but I wish to comment on their differential diagnosis as to drug induced dystonic reactions

Paroxysmal torticollis is not the only condition which recurs with a regular periodicity Drug induced torticollis can also occur with a regular periodicity One of our patients (case 3)—reported in detail in our original paper—presented with a distressing and bizarre condition which we called cyclic torticollis (3) For more than a year this patient suffered from torticollis recurring every one to two months and lasting ? to 4 days Observations on two different occasions in a *department of neurology* did not elucidate the origin of his bizarre disease Sensitized by our previous cases we asked the mother whether her son had been given metoclopramide one of the drugs causing dystonic reactions We were then told that he suffered from cyclic vomiting and that whenever he started vomiting he received this drug A complete discontinuation of metoclopramide rescued the patient from his distressing symptoms'

Drug induced dystonia does not necessary involve the face and mouth Since publication of our paper in 1970 we have seen several patients in whom the paroxysmal torticollis was the only manifestation of drug induced dystonia

Table 1 (2) illustrates that torticollis can indeed be accompanied by trismus and facial *grimacing but that these accompanying symp* toms are not obligatory More frequently the

Table 1 *Symptoms of drug induced dystonia*

Symptoms	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8	Case 9	Case 10
Oculogyric crisis	+	+	+	+		+	+	+	+	+
Retrocollis	+	+	+	+	+	+	+			+
Torticollis	+		+	+		+	+	+	+	+
Neck pain	+	+	+	+		+				
Tremor		+				+	+	+		
Trismus		+				+	+			+
Anxiety	+		+				+			
Dysarthria			+	+						+
Facial grimacing	+	+								+
Perspiration		+				+	+			
Akathisia or motor restlessness	+								+	
Clonic movements of facial mucles neck or back	+					+				
Contr ctures of abdominal and lumbar muscles								+		
Dysphagia					+					
Pain in limbs				+						
Retropelvis							+			

Ref Tijdschr Gastroenterol *14* 05 1971

Acta Pædiatr Scand 68 913–914 1979

SHORT COMMUNICATION

DIAGNOSIS AND THERAPEUTIC STUDIES IN IDIOPATHIC PULMONARY HEMOSIDEROSIS

Idiopathic pulmonary hemosiderosis was diagnosed in two children who presented with marked iron deficiency anemia and were refractory to long term iron therapy The importance of early diagnosis and the beneficial effect of prednisone is discussed

Case I The patient was well until age 2½ years when fatigue pallor coughing and occasional hemoptysis appeared On examination she was pale there were no other abnormal findings The hemoglobin concentration was only 4 8 g/l Iron deficiency anemia was diagnosed and she was treated with blood transfusions and oral iron One year later the symptoms recurred The physical examination was unremarkable except for pallor Chest radiograms showed infiltrations in both lung bases The sputum contained iron laden macrophages Cow s milk hypersensitivity was ruled out by lack of improvement on a milk free diet for about 2 months and a lack of serum precipitins to cow milk proteins She was treated with prednisone (5 mg×4) daily for one and a half years and intermittently (30 mg weekly and 2½ mg every second day) thereafter until she died ACTH (30 I E every 2 weeks and 4 weeks) was given for eight years azathioprin (75 mg daily) was administered thereafter for two years After eight years of disease at age 12 her lung function started to deteriorate She died at the age of 16 years in respiratory failure after several episodes of hemoptysis Autopsy showed marked pulmonary hemosiderosis and pulmonary hemorrhages

Case II She was well until age 3 years when fatigue irritability and episodes of coughing began The hematologic findings were typical of iron deficiency anemia and she was treated with oral and parenteral iron and blood transfusion with only temporary increase in hemoglobin concentration Chest radiograms then showed widespread infiltrations A needle aspiration of the lung was negative but iron stained macrophages were demonstrated repeatedly in laryngeal swabs and gastric washings Lung biopsy confirmed the diagnosis of pulmonary hemosiderosis One gram of dry lung tissue contained 6 87 mg of iron She was without cow s milk for about one year No change of her condition was observed when cow s milk was reintroduced Prednisone (5 mg×4) was given orally for one month and then 20 mg every other day Half a year later the pulmonary symptoms and anemia reappeared Daily dosage prednisone (5 mg×4) was reinstituted for a short period and then changed again to 20 mg every other day Two years after the prednisone therapy was started she is thriving growing well and has no signs of active disease

Comments Only three cases of idiopathic pulmonary hemosiderosis have been reported from Scandinavia (3 5 7) The diagnosis may easily be missed and not made before autopsy (7 8) Although iron therapy should be avoided because it can enhance the deposition of iron in the lung (1 2 6) many cases such as our own are initially treated with oral or parenteral iron or blood transfusions because of severe iron deficiency anemia (4 5 6) In cases of iron deficiency anemia with no response to oral iron and no apparent external blood loss one must consider the possibility of an internal hemorrhage as found in pulmonary hemosiderosis The most important

torticollis is accompanied by retrocollis and oculogyric crises symptoms which are also mentioned in some of the cases reported by the authors

Again I am puzzled by the fact that all four cases reported by Sanner & Bergstrom also present—besides the torticollis—some other symptoms of drug induced dystonia which are mentioned in Table I retrocollis (case 1) oculogyric crisis (case 2) tortipelvis (= curva tion of the trunk in cases 2 3 and 4) tremor (case 2) *perspiration* (case 4) and possibly dysphagia (case 2) For these reasons I wish to refer to my previous statement

It is very important to stress that whenever a patient presents a paroxysmal or cyclic torticollis it can be a symptom of drug induced dystonia (1) The diagnosis of such reactions can be very difficult unless the physician has a high index of suspicion A careful history should be taken every time one is confronted with this symptom searching for the dys tonia inducer and enumerating these drugs for the parents (phenothiazines metoclo pramide haloperidol a o) Indeed it has been our experience that some parents use some of these drugs regularly as a hypnotic as an anti emetic or as a cough syrup for their children and no longer consider them as drugs

I fully agree with the authors that parox ysmal torticollis remains a puzzling condition with many unsolved questions Further care ful observation accurate description of all the accompanying symptoms and a detailed history taking could help in clarifying the entity of this syndrome

Maria Casteels van Daele

Department of Paediatrics
Akademisch Ziekenhuis Gasthuisberg
B 3000 Leuven
Belgium

REFERENCES

1 Casteels van Daele M Paroxysmal torticollis in in fancy *Am J Dis Child* 120 88 1970
2 Casteels van Daele M & Jaeken J Primperan and its side effects *Tijdschr Gastroenterol* 14 05 1971
3 Casteels van Daele M Jaeken J van der Schueren A Zimmerman A & van den Bon P Dystonic reac tions in children caused by metoclopramide *Arch Dis Child* 45 130 1970
4 Sanner G & Bergstrom B Benign paroxysmal tor ticollis in infancy *Acta Paediatr Scand* 68 219 1979

The Editor has asked Dr Sanner to comment on this letter

Sir

It seems quite clear that according to the ex perience of Casteels Van Daele drug induced dystonia must always be held in mind in cases with torticollis even in the absence of facial involvement However the prescription of phenothiazine drugs for small children—and especially infants—seems to be far less fre quent in Sweden than in some other countries

Our four patients had not been given any drugs

Gunnar Sanner

Dept of Paediatrics
Central Hospital
S-651 85 Karlstad
Sweden

Acta Pædiatr Scand 68 915–919 1979

CASE REPORT

PRIMARY MYELOPEROXIDASE DEFICIENCY ASSOCIATED WITH IMPAIRED NEUTROPHIL MARGINATION AND CHEMOTAXIS

C F ROBERTSON Y H THONG G L HODGE and K CHENEY

From the Department of Paediatrics University of Adelaide and the Department of Haematology the Adelaide Children s Hospital North Adelaide South Australia Australia

ABSTRACT Robertson C F Thong Y H Hodge G L and Cheney K (Department of Paediatrics University of Adelaide and Department of Haematology The Adelaide Children s Hospital Adelaide South Australia) Primary myeloperoxidase deficiency associated with impaired neutrophil margination and chemotaxis Acta Paediatr Scand 68 915 1979 —The first infant with myeloperoxidase deficiency is described Impairment of neutrophil margination and chemotaxis may have contributed to recurrent infections

KEY WORDS Myeloperoxidase deficiency chemotaxis neutrophil leucocytes

Primary deficiency of myeloperoxidase (MPO) is a very rare disorder of phagocytic cells first described by Alius & Alius (27) as a coincidental finding in a healthy adult female the levels of the enzyme in her parents sister and four children were all normal In 1963 Grignaschi (8) reported MPO deficiency in two asymptomatic siblings one of these had two children with normal enzyme levels Stendahl (20) described the deficiency in a 46-year-old man who presented with pustular psoriasis this patient had an identical twin who also had pustular psoriasis but died from myocardial infarction prior to the study Lehrer s (13) patient was a 49 year-old man who suffered from chronic bronchitis maturity-onset diabetes mellitus and systemic candidiasis

We report here the first case of MPO deficiency diagnosed in infancy This patient presented with a history of repeated infections The MPO deficiency by itself cannot account entirely for the undue susceptibility to infection Although the interaction between MPO and hydrogen peroxide (H_2O_2) and halide is considered by Klebanoff to be a potent microbicidal mechanism of phagocytic cells (12) this conclusion is not supported by recent studies (10) Moreover most patients with MPO deficiency do not demonstrate a strong predilection towards infectious complications and their neutrophils have only a slight delay in in vitro killing of microorganisms Hence immunological studies were conducted to determine whether an associated immunodeficiency was responsible for this infant s clinical presentation

CASE REPORT

The patient M L T is a white male now 19 months old He was born at term after an uneventful pregnancy with a weight of 3000 g He developed a candida perineal infection at age 3 months this required more than a month of nystatin treatment to clear At age 6 months he had bronchitis Recurrent aphthous ulceration began at this time These were localised to the lateral edges and tip of the tongue with occasional ones on the buccal mucosa They recurred every 3–4 weeks and persisted for 2–3 weeks before healing with scar formation There were 3 episodes of purulent conjunctivitis from age 9–12 months a swab was taken on one occasion and this grew Haemophilus influenzae type b He also had otitis externa at age 10 months and otitis media at age 11 months

There was no family history of increased susceptibility to infection The parents were not consanguineous The mother aged 6 years and father aged 4 years were

point in the management of a patient with pulmonary hemosiderosis appears to be early diagnosis and the avoidance of iron therapy and blood transfusions and the use of corticosteroid therapy. Short term courses of steroids during the active stage of the disease seem to improve these patients (8). It is doubtful, however, whether longterm steroid therapy is of value, as our first case demonstrated.

T. J. Gutteberg, P. J. Moe
Department of Paediatrics
University of Tromsø
9000 Tromsø
Norway

C. E. Norén
Department of Paediatrics
Lasarettet i Sundsvall
Sundsvall
Sweden

REFERENCES

1. Donald K. J., Edwards R. L. & McEvoy J. D. S.: Alveolar capillary basement membrane lesions in Goodpasture's syndrome and idiopathic pulmonary hemosiderosis. *Am J Med 59*: 64? 1975.
2. Donlan C. J., Srodes H. C. & Duffy F. D.: Idiopathic pulmonary hemosiderosis. *Chest 68*: 577, 19 5.
3. Gellerstedt N.: Über die essensielle anaemische Form der braunen Lungeninduration. *Acta Path Scand 16*: 386, 1939.
4. Gillmann P. A. & Zinkman W. H.: Severe idiopathic pulmonary hemosiderosis in the absence of clinical or radiological evidence of pulmonary disease. *J Pediatr 75*: 118, 1963.
5. Halvorsen S.: Cortisone treatment of idiopathic pulmonary hemosiderosis. *Acta Paediatr Scand 45*: 139, 1956.
6. Hyatt R. W., Adelstein E. R., Halazum J. F. & Lukens J. N.: Ultrastructure of the lung in idiopathic pulmonary hemosiderosis. *Am J Med 5? 8??* 19/?.
7. Jacobsen K. B.: Plutselig død ved idiopatisk pulmonal hemosiderose. *Nord Med 86*: 978, 1971.
8. Soergel K. H. & Sommers S. C.: Idiopathic pulmonary hemosiderosis and related syndromes. *Am J Med 32*: 499, 1962.

Acta Pædiatr Scand 68 915–919 1979

CASE REPORT

PRIMARY MYELOPEROXIDASE DEFICIENCY ASSOCIATED WITH IMPAIRED NEUTROPHIL MARGINATION AND CHEMOTAXIS

C F ROBERTSON Y H THONG G L HODGE and K CHENEY

From the Department of Paediatrics University of Adelaide and the Department of Haematology the Adelaide Children s Hospital North Adelaide South Australia Australia

ABSTRACT Robertson C F Thong Y H Hodge G L and Cheney K (Department of Paediatrics University of Adelaide and Department of Haematology The Adelaide Children s Hospital Adelaide South Australia) Primary myeloperoxidase deficiency associated with impaired neutrophil margination and chemotaxis Acta Paediatr Scand 68 915 1979 —The first infant with myeloperoxidase deficiency is described Impairment of neutrophil margination and chemotaxis may have contributed to recurrent infections

KEY WORDS Myeloperoxidase deficiency chemotaxis neutrophil leucocytes

Primary deficiency of myeloperoxidase (MPO) is a very rare disorder of phagocytic cells first described by Alius & Alius (27) as a coincidental finding in a healthy adult female the levels of the enzyme in her parents sister and four children were all normal In 1963 Grignaschi (8) reported MPO deficiency in two asymptomatic siblings one of these had two children with normal enzyme levels Stendahl (20) described the deficiency in a 46 year old man who presented with pustular psoriasis this patient had an identical twin who also had pustular psoriasis but died from myocardial infarction prior to the study Lehrer s (13) patient was a 49 year-old man who suffered from chronic bronchitis maturity-onset diabetes mellitus and systemic candidiasis

We report here the first case of MPO deficiency diagnosed in infancy This patient presented with a history of repeated infections The MPO deficiency by itself cannot account entirely for the undue susceptibility to infection Although the interaction between MPO and hydrogen peroxide (H_2O_2) and halide is considered by Klebanoff to be a potent microbicidal mechanism of phagocytic cells (12) this conclusion is not supported by recent studies (10) Moreover most patients with MPO deficiency do not demonstrate a strong predilection towards infectious complications and their neutrophils have only a slight delay in in vitro killing of microorganisms Hence immunological studies were conducted to determine whether an associated immunodeficiency was responsible for this infant s clinical presentation

CASE REPORT

The patient M L T is a white male now 19 months old He was born at term after an uneventful pregnancy with a weight of 3000 g He developed a candida perineal infection at age 3 months this required more than a month of nystatin treatment to clear At age 6 months he had bronchitis Recurrent aphthous ulceration began at this time These were localised to the lateral edges and tip of the tongue with occasional ones on the buccal mucosa They recurred every 3–4 weeks and persisted for 2–3 weeks before healing with scar formation There were 3 episodes of purulent conjunctivitis from age 9–1? months a swab was taken on one occasion and this grew Haemophilus influenzae type b He also had otitis externa at age 10 months and otitis media at ag 11 months

There was no family history of increased susceptibility to infection The parents were not consanguineous The mother aged 6 years and father aged ?4 years were

point in the management of a patient with pulmonary hemosiderosis appears to be early diagnosis and the avoidance of iron therapy and blood transfusions and the use of corticosteroid therapy. Short term courses of steroids during the active stage of the disease seem to improve these patients (8). It is doubtful, however, whether longterm steroid therapy is of value as our first case demonstrated.

T. J. Gutteberg, P. J. Moe

Department of Pediatrics
University of Tromsø
9000 Tromsø
Norway

C. I. Norén

Department of Pediatrics
Lasarettet i Sundsvall
Sundsvall
Sweden

REFERENCES

1. Donald K J, Edwards R L & McEvoy J D S. Alveolar capillary basement membrane lesions in Goodpasture's syndrome and idiopathic pulmonary hemosiderosis. *Am J Med* 59: 642, 1975.
2. Donlan C J, Srodes H C & Duffy F D. Idiopathic pulmonary hemosiderosis. *Chest* 68: 577, 1975.
3. Gellerstedt N. Über die essensielle anaemische Form der braunen Lungeninduration. *Acta Path Scand* 16: 386, 1939.
4. Gillmann P A & Zinkman W H. Severe idiopathic pulmonary hemosiderosis in the absence of clinical or radiological evidence of pulmonary disease. *J Pediatr* 75: 118, 1963.
5. Halvorsen S. Cortisone treatment of idiopathic pulmonary hemosiderosis. *Acta Paediatr Scand* 45: 139, 1956.
6. Hyatt R W, Adelstein E R, Halazum J F & Lukens J N. Ultrastructure of the lung in idiopathic pulmonary hemosiderosis. *Am J Med* 52: 822, 1972.
7. Jacobsen K B. Plutselig død ved idiopatisk pulmonal hemosiderose. *Nord Med* 86: 978, 1971.
8. Soergel K H & Sommers S C. Idiopathic pulmonary hemosiderosis and related syndromes. *Am J Med* 32: 499, 1962.

Acta Pædiatr Scand 68 915-919 1979

CASE REPORT

PRIMARY MYELOPEROXIDASE DEFICIENCY ASSOCIATED WITH IMPAIRED NEUTROPHIL MARGINATION AND CHEMOTAXIS

C F ROBERTSON Y H THONG G L HODGE and K CHENEY

F om the Department of Paediatrics Unn ersity of Adelaide and the Depart nent of Haematology the Adelaide Children s Hospital North Adelaide South Australia Australia

ABSTRACT Robertson C F Thong Y H Hodge G L and Cheney K (Department of Paediatrics University of Adelaide and Department of Haematology The Adelaide Children s Hospital Adelaide South Australia) Primary myeloperoxidase deficiency associated with impaired neutrophil margination and chemotaxis Acta Paediatr Scand 68 915 1979 —The first infant with myeloperoxidase deficiency is described Impairment of neutrophil margination and chemotaxis may have contributed to recurrent infections

KEY WORDS Myeloperoxidase deficiency chemotaxis neutrophil leucocytes

Primary deficiency of myeloperoxidase (MPO) is a very rare disorder of phagocytic cells first described by Alius & Alius (27) as a coinci dental finding in a healthy adult female the levels of the enzyme in her parents sister and four children were all normal In 1963 Grig naschi (8) reported MPO deficiency in two asymptomatic siblings one of these had two children with normal enzyme levels Stendahl (?0) described the deficiency in a 46 year-old man who presented with pustular psoriasis this patient had an identical twin who also had pustular psoriasis but died from myocardial infarction prior to the study Lehrer s (13) patient was a 49 year old man who suffered from chronic bronchitis maturity onset dia betes mellitus and systemic candidiasis

We report here the first case of MPO defi ciency diagnosed in infancy This patient pre sented with a history of repeated infections The MPO deficiency by itself cannot account entirely for the undue susceptibility to infec tion Although the interaction between MPO and hydrogen peroxide (H_2O) and halide is considered by Klebanoff to be a potent micro bicidal mechanism of phagocytic cells (12) this conclusion is not supported by recent studies (10) Moreover most patients with MPO deficiency do not demonstrate a strong predilection towards infectious complications and their neutrophils have only a slight delay in in vitro killing of microorganisms Hence immunological studies were conducted to determine whether an associated immuno deficiency was responsible for this infant s clinical presentation

CASE REPORT

The patient M L T is a white male now 19 months old He was born at term after an uneventful pregnancy with a weight of 3 000 g He developed a candida perineal infec tion at age 3 months this required more than a month of nystatin treatment to clear At age 6 months he had bronchitis Recurrent aphthous ulceration began at this time These were localised to the lateral edges and tip of the tongue with occasional ones on the buccal mucosa They recurred every 3–4 weeks and persisted for ?–3 weeks before healing with scar formation Th re were 3 episodes of purulent conjunctivitis from age 9–1 months a swab was taken on one occasion and this grew Haemo philus influenzae type b He also had otitis externa at age 10 months and otitis media at age 11 months

There was no family history of increased susceptibility to infection The parents were not consanguineous The mother aged 6 years and father aged ?4 years were

Table 1 *Immunological screening tests on an infant with myeloperoxidase deficiency*

Test	Results	Normal range
Neutrophil function		
Marrow pool	3 345/µl increase	>2 000
Margination pool	22 7% increase	>45%
Bactericidal	87 5%	
	(% *S aureus* killed in 2 h)	70–90
Fungicidal	72 0%	
	(% *T glabrata* killed in 4 h)	75–90%
Hexose monophosphate shunt activity	5 9	
	(stimulation index)	>3 0
Quantitative iodination	1 2	
	(pmols/10⁷ cells/h)	>3 0
Chemotaxis	0 61±0 33	1 26±0 07
Serum immunoglobulins		
IgG	7 5 g/l	2 6–13 9
IgA	*0 45 g/l*	*0 2–1 2*
IgM	0 45 g/l	0 3–1 5
IgE	3 4 U/ml	64–392
Serum complement		
C3	1 74 g/l	0 65–1 50
C4	0 29 g/l	0 20–0 40
CH50	128 U/ml	95–165
T-cells	68%	50–70
B-cells	11%	5–15
Lymphocyte transformation[b]		
Phytohaemagglutinin	41 498 cpm	>10 000
Pokeweed mitogen	13 658 cpm	>5 000
Concanavalin A	29 907 cpm	>10 000

Mean ± S D of 4 experiments ($p<0\ 01$) on 4 different occasions
[b] Expressed as counts per minute (cpm) ^{3}H thymidine uptake in stimulated cultures resting cultures showed uptake of 519 cpm

healthy The only sibling a male aged 4 years was also healthy

Physical examination at age 12 months revealed a well nourished infant weighing 9 85 kg (25–50%) and measuring 73 0 cm in height (25–50 %) Development milestones were normal The haemoglobin was 10 5 g/dl platelet count 150 000/µl ¹ and white cell count 11 600/µl ¹ with 29% neutrophils 1% eosinophils 68 % lymphocytes 2% monocytes *The patient was admitted to the Adelaide* Children s Hospital at 12 months of age for immunological investigations during this time he was clinically well and free from infection

MATERIALS AND METHODS

In order to exclude cyclic neutropenia absolute neutrophil counts were performed twice weekly over 6 weeks The adequacy of the marrow neutrophil reserve was tested by doing an absolute neutrophil count by finger prick at 0 and 5 hours after intravenous injection of 50 mg hydrocortisone (5) The neutrophil margination pool was assessed at 0 and 1 hour after the subcutaneous injection of *0 1 ml* 1 1000 solution of adrenaline (18)

The serum immunoglobulins (A G M) and complement (C3 C4) concentrations were measured by radial immuno diffusion using commercial plates (Behringwerke W Germany) serum IgE by the Phadebas test kit (Pharmacia Sweden) total haemolytic complement by the lysis of sheep red cells (15)

Lymphocytes and neutrophils were purified from heparinised blood by a one step gradient sedimentation proce

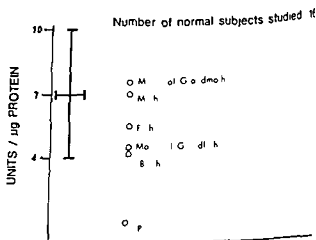


Fig 1 Quantitative assay of myeloperoxidase activity in leukocytes

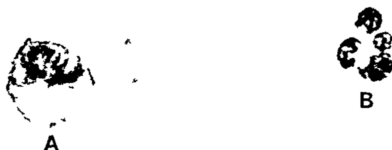


Fig 2 Peripheral blood smear of patient showing positive staining for myeloperoxidase in eosinophil (A) but not not neutrophil (B)

dure (6) T-cells enumerated by spontaneous rosetting with heep red cells (19) lymphocyte transformation to mitogens by a micro-culture method (4) neutrophil hexose-monophosphate shunt activity by conversion of ${}^{14}C$ 1-gluco e to ${}^{14}CO_2$ (3) quantitative iodination by a mi roassay technique (6) bactericidal capacity by reduction of *Staphylococcus au eus* colony counts (?1) fungicidal capacity by reduction of *Torulopsis glabrata* colony counts ( ) and chemotaxis by migration under agarose (5)

White blood ells were stained for peroxidase by a mod fication of the method of Goodpasture (7) u ing toluidine as substrate Myeloperoxidase activity was a sayed by the method of Klebanoff (11) with slight modific tion (13) using (anisidine a substrate

RESULTS

Twice weekly white cell counts over 6 weeks showed that absolute neutrophil numbers were consistently over 2000/μl thereby excluding the possibility of cyclic neutropaenia The results of immunological screening tests are presented in Table 1 All tests were normal except for impaired neutrophil margination chemotaxis and quantitative iodination

Quantitative studies revealed normal MPO concentrations in family members (Fig 1) Peripheral blood smear of the patient (Fig 2) confirmed the deficiency of MPO in his neutrophils but not eosinophils Family studies showed that brother and mother have normal neutrophil iodination (3 4 and 3 7 pmols/10^7 cells/h respectively) and chemotaxis (0 9 and 1 15 mm/2 h respectively) When neutrophil bactericidal and fungicidal assays were performed at varying incubation times a delay in killing of fungi (Fig 3) but not bacteria (Fig 4) was observed compared to other family members and controls

DISCUSSION

Neutrophils are important mediators of resistance to infection (2 3) They are the first cells

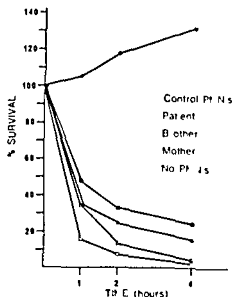


Fig. 3. Fungicidal capacity of neutrophils in relation to incubation time.

to gather at the site of inflammation where they ingest and kill microorganisms. The process involves mobilization of these cells from marrow stores, the deployment to the lining of the blood vessels (margination pool) and their final egress into tissues by means of chemotaxis. It has been shown that two to four hours after bacterial invasion is the most critical period for the inflammatory response to control infection (16). Hence patients with chemotactic disorders are invariably subjected to infectious complications (2, 3, 28). The results of the present studies indicate the impairment of neutrophil margination and chemotaxis may be responsible for the recurrent infections in our patient. The associated MPO deficiency may also be contributory to his problems, although this conclusion must be tentative in the light of recent findings that the MPO-H_2O_2-halide system is not directly microbicidal. Others (17) have shown that iodination of microbial membranes can be inhibited by ascorbate without a concomitant decrease in killing. Moreover, addition of superoxide dismutase increases iodination but decreases killing (1, 9). In vitro studies have shown only a slight delay in the killing of microorganisms (13) and this is confirmed by our data for fungi but not bacteria. Thus the MPO-H_2O-halide system may play only a minor role to the other microbicidal mechanisms such as superoxide anion, cationic proteins, lysozyme and lactoferrin (12).

Secondary MPO deficiency has been described in association with haematological disorders (4, 14) but their infectious complications have been attributed to MPO deficiency rather than debilitation and immunodepression. Of the 6 cases of primary MPO deficiency previously reported only 2 developed infectious complications in adulthood. One had *diabetes mellitus* and *systemic* candidiasis (13), while the other had pustular psoriasis (20). In the latter situation the dermatological condition might have been the predisposing cause of the skin infection. It would appear that MPO deficiency per se may not predispose to recurrent infection. Our patient is unique in presenting with recurrent infections in infancy, and this can be explained on the basis of a *defect in neutrophil* margination and chemotaxis.

The MPO enzyme is present in a wide variety of animal species (12). Several isoenzymes are known to exist. The enzyme of neutrophils and monocytes is different from that in eosinophils, hence the positive staining for MPO in eosinophils of our patient al

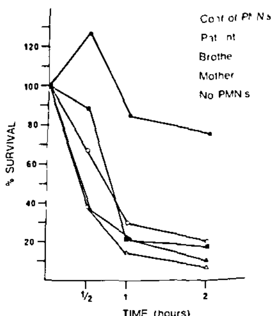


Fig. 4. Bactericidal capacity of neutrophils in relation incubation time.

though MPO deficiency in neutrophils has been demonstrated by quantitative iodination enzyme assay and direct staining The gene for neutrophil MPO deficiency is believed to be transmitted by an autosomal recessive mode The heterozygous state cannot be diag nosed by enzyme assay because parents do not show intermediate values

REFERENCES

1 Baehner R L Murrmann S K Davis J & Johns ton R B Jr The role of superoxide anion and hydro gen peroxide in phagocytosis associated oxidative metabolic reactions *J Clin Invest* 56 571 1975
Bellanti J A & Dayton D H *The phagocytic cell in host resistance* Raven Press New York 1975
3 Bjorksten B & Quie P G Abnormalities of cir culating phagocyte function *In* R A Thompson (ed) *Recent advances in clinical immunology* Chur chill Livingstone Edinburgh 1978 vol 1 p 187
4 Davies A T Brunning R T & Quie P G Poly morphonuclear leukocyte myeloperoxidas defi ciency in a patient with myelomonocytic leukaemia *N Engl J Med* 285 789 1971
5 Dienard A S Fortuny I F Theologides A Anderson G L Boer J & Kennedy B J Studies on the neutropenia of cancer chemotherapy *Cancer* 33 1210 1974
6 Ferrante A & Thong Y H A rapid one step pro cedure for purification of mononuclear and polymor phonuclear leukocytes from human blood using a modification of the hypaqueficoll technique *J Immu nol Methods* 24 389 1978
7 Goodpasture E W A peroxidase rea tion with sodium nitroprusside and benzidine in blood smears and tissues *J Lab Clin Med* 4 44 1919
8 Grignaschi V J Sperperato A M & Etcheverry M J Un nuevo cuadro citoquimico negatividad espontanea de las reacciones de peroxidasas oxi dasas y lipido en la progenie neutrofila y en los mono citos de dos hermanos *Rev Assoc Med Argent* 77 18 1971
9 Johnston R B Jr Keele B B Jr Misra H P Lehmeyer J E Webb L S Baehner R L & Rajagopalan K V The role of superoxide anion generation in phagocytic bactericidal activity *J Clin Invest* 55 1357 1975
10 Johnson R B Jr Oxygen metabolism and the micro bicidal activity of macrophages *Federation Proceedings* 37 759 1978
11 Klebanoff S J Inactivation of estrogen by rat uterine preparation *Endocrinology* 76 301 1965
1 Klebanoff S J Antimicrobial systems in neutro philic polymorph nuclear leukocytes *Semin Hema t l* 117 1975
13 Lehrer R I & Cline M J Leukocyte myeloperoxi dase deficiency and di seminated candidiasis the role of myeloperoxidase in resistance to *candida* infec infection *J Clin Invest* 48 1478 1969
14 Lehrer R I Goldberg L S Apple M & Rosen thal N P Refractory megaloblastic anemia with myeloperoxidase deficient neutrophils *Ann Int Med* 76 447 197
15 Mayer M M Complement and complement fixa tion *In* E A Kabat (ed) *Experimental immuno chemistry* Charles C Thomas Springfield Ill 1971 2nd ed p 133
16 Miles A Z Miles E M & Burke J The value and duration of defence reactions of the skin to pri mary lodgement of bacteria *Br J Exp Pathol* 38 79 1957
17 McColl C E DeChatelet L R Cooper M R & Ashburn P The effect of ascorbid acid on bacteri cidal mechanisms of neutrophils *J Infect Dis* 124 194 1971
18 Samuels A J Primary and secondary leukocyte changes following the intramuscular injection of epinephrine hydrochloride *J Clin Invest* 30 941 1951
19 Scheinberg M Blacklow N R Goldstein A L Parrino J A Rose F B & Cathcart E S In fluenza response of T-cell lymphopenia to thymosin *N Engl J Med* 294 1208 1976
0 Stendahl O & Lundgren S Function of granulo cytes with deficient myeloperoxidase mediated iodi nation in a patient with generalized pustular psoriasis *Scand J Haematol* 16 144 1976
21 Thong Y H Ferrante A & Ness D Neutrophil phagocytic and bactericidal hysfunction induced by bilirubin *Aust Paediatr J* 13 287 1977
22 Thong Y H & Ferrante A Phagocytosis and kill ing of *Torulopsis glabrata* by human neutrophils *IRCS Med Sci* 5 482 1977
23 Thong Y H & Rencis V Bilirubin inhibits hexose monophosphate shunt activity of phagocytosing neutrophils *Acta Paediatr Scand* 66 757 1977
24 Thong Y H & Ferrante A Inhibition of mitogen induced human lymphocyte proliferative responses by quinine *Am J Trop Med Hyg* 27 354 1978
25 Thong Y H Douglas B S & Ferrante A Ab normal neutrophil chemotaxis in a syndrome of un usual facies proportionate small stature and sensori neural deafness mutism *Acta Paediatr Scand* 67 383 1978
6 Thong Y H & Ferrante A A semi automated microassay te hnique for quantitative neutrophil iodi nation *J Immunol Methods* 20 297 1978
27 Undritz E Die Alius-Grignaschi Anomalie der erblichkonstitutionelle Peroxydasedefekt der Neutro philen und Monozyten *Blut* 14 129 1966
8 Ward P A Leukotaxis and leukotactic disorders A review *Am J Pathol* 77 520 1974

Submitted March 1 1979
Accepted April 4 1979

(Y H T) Dept Paediatrics University of Adelaide
The Adelaide Children s Hospital
North Adelaide 5006 South Australia
Australia

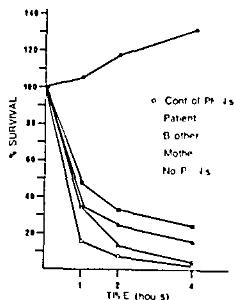


Fig. 3. Fungicidal capacity of neutrophils in relation to incubation time.

to gather at the site of inflammation where they ingest and kill microorganisms. The process involves mobilization of these cells from marrow stores, the deployment to the lining of the blood vessels (margination pool) and their final egress into tissues by means of chemotaxis. It has been shown that two to four hours after bacterial invasion is the most critical period for the inflammatory response to control infection (16). Hence patients with chemotactic disorders are invariably subjected to infectious complications (2, 3, 28). The results of the present studies indicate the impairment of neutrophil margination and chemotaxis may be responsible for the recurrent infections in our patient. The associated MPO deficiency may also be contributory to his problems, although this conclusion must be tentative in the light of recent findings that the MPO-H_2O_2-halide system is not directly microbicidal. Others (17) have shown that iodination of microbial membranes can be inhibited by ascorbate without a concomitant decrease in killing. Moreover, addition of superoxide dismutase increases iodination but decreases killing (1, 9). In vitro studies have shown only a slight delay in the killing of microorganisms (13) and this is confirmed by our data for fungi but not bacteria. Thus the MPO-H_2O_2-halide system may play only a minor role to the other microbicidal mechanisms such as superoxide anion, cationic proteins, lysozyme and lactoferrin (12).

Secondary MPO deficiency has been described in association with haematological disorders (4, 14) but their infectious complications have been attributed to MPO deficiency rather than debilitation and immunodepression. Of the 6 cases of primary MPO deficiency previously reported only 2 developed infectious complications in adulthood. One had diabetes mellitus and systemic candidiasis (13), while the other had pustular psoriasis (20). In the latter situation the dermatological condition might have been the predisposing cause of the skin infection. It would appear that MPO deficiency per se may not predispose to recurrent infection. Our patient is unique in presenting with recurrent infections in infancy, and this can be explained on the basis of a defect in neutrophil margination and chemotaxis.

The MPO enzyme is present in a wide variety of animal species (12). Several isoenzymes are known to exist. The enzyme of neutrophils and monocytes is different from that in eosinophils, hence the positive staining for MPO in eosinophils of our patient al-

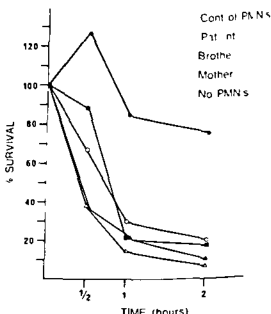


Fig. 4. Bactericidal capacity of neutrophils in relation incubation time.

though MPO deficiency in neutrophils has been demonstrated by quantitative iodination enzyme assay and direct staining The gene for neutrophil MPO deficiency is believed to be transmitted by an autosomal recessive mode The heterozygous state cannot be diag nosed by enzyme assay because parents do not show intermediate values

REFERENCES

1 Baehner R L Murrmann S K Davis J & Johns ton R B Jr The role of superoxide anion and hydro gen peroxide in phagocytosis associated oxidative metabolic reactions J Clin In est 56 571 1975

2 Bellanti J A & Dayton D H The phagocytic cell in host resistance Raven Press New York 1975

3 Bjorksten B & Quie P G Abnormalities of cir culating phagocyte function In R A Thompson (ed) Recent advances in clinical immunology Chur chill Livingstone Edinburgh 1978 vol 1 p 18?

4 Davies A T Brunning R T & Quie P G Poly morphonuclear leukocyte myeloperoxidas defi ciency in a patient with myelomonocytic leukaemia N Engl J Med 285 789 1971

5 Dienard A S Fortuny I F Theologides A Anderson G L Boer J & Kennedy B J Studies on the neutropenia of cancer chemotherapy Cancer 33 1?10 1974

6 Ferrante A & Thong Y H A rapid one step pro cedure for purification of mononuclear and polymor phonuclear leukocytes from human blood using a modification of the hypaqueficoll technique J Immu nol Methods 24 389 1978

7 Goodpasture E W A peroxidase reaction with sodium nitroprusside and benzidine in blood smears and tissue J Lab Clin Med 4 44? 1919

8 Gngnaschi V J Sperperato A M & Etcheverry M J Un nuevo cuadro citoquimico negatividad espontanea de las reacciones de peroxidasas oxi dasas y l pido en la progenie neutrofila y en los mono citos de dos hermanos Re Assoc Med Argent 77 18 1971

9 Johnston R B Jr Keele B B Jr Misra H P Lehmeyer J E Webb L S Baehner R L & Rajagopalan K V The role of superoxide anion generation in phagocytic bacteri idal activity J Clin Inve t 55 1357 1975

10 Johnson R B Jr Oxygen metabolism and the micro bicid l activity of macrophages Federal n Pro ceedings 37 ?759 1978

11 Klebanoff S J Inactivation of estrogen by rat uterine preparation End crin l gy 76 301 1965

1 Klebanoff S J Antimicrobial systems in neutro philic polymorphonuclear leukocytes Sem n Hema t l 1? 117 1975

13 Lehrer R I & Cline M J Leukocyte myeloperoxi dase deficiency and disseminated cand d asis the role of myeloperoxidase in resistance to candi la infec infection J Clin Invest 48 1478 1969

14 Lehrer R I Goldberg L S Apple M & Rosen thal N P Refractory megaloblastic anemia with myeloperoxidase deficient neutrophils Ann Int Med 76 447 197?

15 Mayer M M Complement and complement fixa tion In E A Kabat (ed) Experimental immuno chemistry Charles C Thomas Springfield Ill 1971 ?nd ed p 133

16 Miles A Z Miles E M & Burke J The value and duration of defence reactions of the skin to pri mary lodgement of bacteria Br J Exp Pathol 38 79 1957

17 McColl C E DeChatelet L R Cooper M R & Ashburn P The effect of ascorbid acid on bacteri cidal mechanisms of neutrophils J Infect Dis 124 194 1971

18 Samuels A J Primary and secondary leukocyte changes following the intramuscular injection of epinephrine hydrochloride J Clin Invest 30 941 1951

19 Scheinberg M Blacklow N R Goldstein A L Parrino J A Rose F B & Cathcart E S In fluenza response of T-cell lymphopenia to thymosin N Engl J Med ?94 1?08 1976

0 Stendahl O & Lundgren S Function of granulo cytes with deficient myeloperoxidase mediated iodi nation in a patient with generalized pustular psoriasis Scand J Haematol 16 144 19/6

?1 Thong Y H Ferrante A & Ness D Neutrophil phagocytic and bactericidal hysfunction induced by bilirubin Aust Paediat J 13 87 1977

Thong Y H & Ferrante A Phagocyto is and kill ing of Torulopsis glabrata by human neutrophils IRCS Med Sci 5 48? 1977

?3 Thong Y H & Rencis V Bilirubin inhibits hexose monophosphate shunt activity of phagocytosing neutrophils Acta Paediatr Scand 66 757 1977

4 Thong Y H & Ferrante A Inhibition of mitogen induced human lymphocyte proliferative responses by quinine Am J Trop Med Hyg 27 354 1978

5 Thong Y H Douglas B S & Ferrante A Ab normal neutrophil chemotaxis in a syndrome of un usual facies proportionate small stature and sensori neural deafness mutism Acta Paediatr Scand 67 383 1978

6 Thong Y H & Ferrant A A semi automated microassay technique for quantitative neutrophil iodi nation J Immunol Meth ds 0 ?97 1978

?7 Undritz E Die Alius-Gngnaschi Anomalie der erblichkonstitutionelle Peroxydasedefekt der Neutro philen und Monozyten Blut 14 1?9 1966

8 Ward P A Leukotaxis and leukotactic disorders A review Am J Path l 77 5?0 1974

Submitted March 1? 1979
Accepted April 4 1979

(Y H T) Dept Paediatrics University of Adelaide
The Adelaide Children s Hospital
North Adelaide 5006 South Australia
Australia

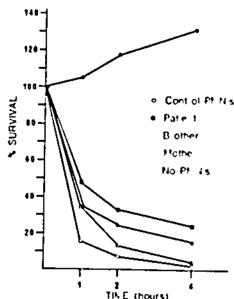


Fig. 3 Fungicidal capacity of neutrophils in relation to incubation time

to gather at the site of inflammation where they ingest and kill microorganisms The process involves mobilization of these cells from marrow stores the deployment to the lining of the blood vessels (margination pool) and their final egress into tissues by means of chemotaxis It has been shown that two to four hours after bacterial invasion is the most critical period for the inflammatory response to control infection (16) Hence patients with chemotactic disorders are invariably subjected to infectious complications (2 3 28) The results of the present studies indicate the impairment of neutrophil margination and chemotaxis may be responsible for the recurrent infections in our patient The associated MPO deficiency may also be contributory to his problems although this conclusion must be tentative in the light of recent findings that the MPO-H O halide system is not directly microbicidal Others (17) have shown that iodination of microbial membranes can be inhibited by ascorbate without a concomitant decrease in killing Moreover addition of superoxide dismutase increases iodination but decreases killing (1 9) In vitro studies have shown only a slight delay in the killing of microorganisms (13) and this is confirmed by our data for fungi but not bacteria Thus the MPO-H O halide system may play only a minor role to the other microbicidal mechanisms such as superoxide anion cationic proteins lysozyme and lactoferrin (12)

Secondary MPO deficiency has been described in association with haematological disorders (4 14) but their infectious complications have been attributed to MPO deficiency rather than debilitation and immunodepression Of the 6 cases of primary MPO deficiency previously reported only 2 developed infectious complications in adulthood One had diabetes mellitus and systemic candidiasis (13) while the other had pustular psoriasis (20) In the latter situation the dermatological condition might have been the predisposing cause of the skin infection It would appear that MPO deficiency per se may not predispose to recurrent infection Our patient is unique in presenting with recurrent infections in infancy and this can be explained on the basis of a defect in neutrophil margination and chemotaxis

The MPO enzyme is present in a wide variety of animal species (12) Several isoenzymes are known to exist The enzyme of neutrophils and monocytes is different from that in eosinophils hence the positive staining for MPO in eosinophils of our patient al

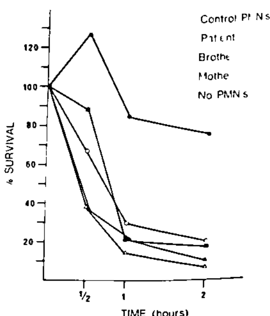


Fig. 4 Bactericidal capacity of neutrophils in relation incubation time

Acta Pædiatr Scand 68 921–922 1979

CASE REPORT

HEMIHYPERTROPHY WITH UNILATERAL FOLLICULITIS AND ACNE

P KAAPA and P SUSITAIVAL

From the Departments of Paediatrics and Dermatology Central Hospital of North Karelia Joensuu Finland

ABSTRACT Kaapa P and Susitaival P (Departments of Paediatrics and Dermatology Central Hospital of North Karelia Joensuu Finland) Hemihypertrophy with unilateral folliculitis and acne Acta Paediatr Scand 68 921 1979 —A case of congenital hemihypertrophy right sided folliculitis and acne in a 16-year-old boy is described Except for hemihypertrophy and skin changes on the right side no other abnormalities were found A similar combination could not be found in the literature

KEY WORDS Hemihypertrophy folliculitis acne

Hemihypertrophy is a rare congenital developmental anomaly a unilateral enlargement of one half of the body either complete or partial Its etiology is not known

Ringrose et al (6) reported changes in skin colour texture secretion and temperature with unusual naevi and teleangiectasiae in 47% of their cases of hemihypertrophy Mental deficiency is found in 28% of the cases There is also a high correlation between renal (Wilms s) (1 2 4) adrenal (5) and hepatic (3) tumours and hemihypertrophy We report a case of congenital hemihypertrophy with acne and folliculitis limited to the hypertrophic side

CASE REPORT

A 16-year-old boy was seen by us because of folliculitis in the scalp and acne in the upper parts of the trunk both on the right side only According to the patient s history his birth was normal after an uneventful pregnancy His birth weight was 4450 g and the Apgar score 10/10 The boy s development was normal At the age of 3 months the mother noticed a difference in temperature between the boy hands the right being warmer than the left The difference in size between the two sides of his body was noticed later but did not receive much attention

The patient s uncle is said to have large hands and one side of the thorax more elevated but we lack further evidence of hemihypertrophy in his case The other members of the family are healthy

Physical examination revealed a healthy school boy who measured 180 5 cm in height and 63 kg in weight being at the eighty fourth percentile in the height chart and at the fiftieth in the weight chart for Finnish boys The right side of the body was darker in colour and felt warmer than the left even the superficial veins were more prominent to the right especially on the right ankle Clinically there were no signs of an AV shunt The forehead and the cheekbones were more prominent on the right side and the right shoulder was elevated The extremities measured 1 cm more in length on the right side The maximum difference in circumference of the two sides was 1 5 cm (biceps and thigh) These differences were also demonstrable in radiographs of the extremities Both sides showed a retardation of 2 to 3 years in bone age slightly more on the left side The boy s pubertal stage was expressed as G5 and P5 according to Tanner (1962)

On the right side of the patient s head there was mild folliculitis in two areas one over the ear and the other extending from the forehead over the scalp Both areas were constant and about 2 cm broad There was also some acne with multiple comedones and a mild inflammatory reaction on the right upper thorax New lesions were erupting continuously

DISCUSSION

In the present case the only abnormalities were hemihypertrophy with accompanying skin changes Acne and folliculitis were the boy s chief complaints The enlargement of his right arm and leg though previously noted had not been considered severe enough for consulting a doctor Acne was noticed at the age of 14 and is though one sided a problem for the patient The peculiar arrangement of

Acta Pædiatr Scand 68 921–922 1979

CASE REPORT

HEMIHYPERTROPHY WITH UNILATERAL FOLLICULITIS AND ACNE

P KAAPA and P SUSITAIVAL

From the Departments of Paediatrics and Dermatology Central Hospital of North Karelia Joensuu Finland

ABSTRACT Kaapa P and Susitaival P (Departments of Paediatrics and Dermatology Central Hospital of North Karelia Joensuu Finland) Hemihypertrophy with unilateral folliculitis and acne Acta Paediatr Scand 68 921 1979 —A case of congenital hemihypertrophy right sided folliculitis and acne in a 16-year old boy is described Except for hemihypertrophy and skin changes on the right side no other abnormalities were found A similar combination could not be found in the literature

KEY WORDS Hemihypertrophy folliculitis acne

Hemihypertrophy is a rare congenital developmental anomaly a unilateral enlargement of one half of the body either complete or partial Its etiology is not known

Ringrose et al (6) reported changes in skin colour texture secretion and temperature with unusual naevi and teleangiectasiae in 47% of their cases of hemihypertrophy Mental deficiency is found in 28% of the cases There is also a high correlation between renal (Wilms s) (1 2 4) adrenal (5) and hepatic (3) tumours and hemihypertrophy We report a case of congenital hemihypertrophy with acne and folliculitis limited to the hypertrophic side

CASE REPORT

A 16-year-old boy was seen by us because of folliculitis in the scalp and acne in the upper parts of the trunk both on the right side only According to the patient s history his birth was normal after an uneventful pregnancy His birth weight was 4450 g and the Apgar score 10/10 The boy s development was normal At the age of 3 months the mother noticed a difference in temperature between the boy s hands the right being warmer than the left The difference in size between the two sides of his body was noticed later but did not receive much attention

The patient s uncle is said to have large hands and one side of the thorax more elevated but we lack further evidence of hemihypertrophy in his case The other members of the family are healthy

Physical examination revealed a healthy school boy who measured 180 5 cm in height and 63 kg in weight being at the eighty fourth percentile in the height chart and at the fiftieth in the weight chart for Finnish boys The right side of the body was darker in colour and felt warmer than the left even the superficial veins were more prominent to the right especially on the right ankle Clinically there were no signs of an AV shunt The forehead and the cheekbones were more prominent on the right side and the right shoulder was elevated The extremities measured 1 cm more in length on the right side The maximum difference in circumference of the two sides was 1 5 cm (biceps and thigh) These differences were also demonstrable in radiographs of the extremities Both sides showed a retardation of 2 to 3 years in bone age slightly more on the left side The boy s pubertal stage was expressed as G5 and P5 according to Tanner (1962)

On the right side of the patient s head there was mild folliculitis in two areas one over the ear and the other extending from the forehead over the scalp Both areas were constant and about 2 cm broad There was also some acne with multiple comedones and a mild inflammatory reaction on the right upper thorax New lesions were erupting continuously

DISCUSSION

In the present case the only abnormalities were hemihypertrophy with accompanying skin changes Acne and folliculitis were the boy s chief complaints The enlargement of his right arm and leg though previously noted had not been considered severe enough for consulting a doctor Acne was noticed at the age of 14 and is though one sided a problem for the patient The peculiar arrangement of

Acta Pædiatr Scand 68 921–922 1979

CASE REPORT

HEMIHYPERTROPHY WITH UNILATERAL FOLLICULITIS AND ACNE

P KAAPA and P SUSITAIVAL

From the Departments of Paediatrics and Dermatology Central Hospital of North Karelia Joensuu Finland

ABSTRACT Kaapa P and Susitaival P (Departments of Paediatrics and Dermatology Central Hospital of North Karelia Joensuu Finland) Hemihypertrophy with unilateral folliculitis and acne Acta Paediatr Scand 68 921 1979.—A case of congenital hemihypertrophy right-sided folliculitis and acne in a 16-year-old boy is described Except for hemihypertrophy and skin changes on the right side no other abnormalities were found A similar combination could not be found in the literature

KEY WORDS Hemihypertrophy folliculitis acne

Hemihypertrophy is a rare congenital developmental anomaly a unilateral enlargement of one half of the body either complete or partial Its etiology is not known

Ringrose et al (6) reported changes in skin colour texture secretion and temperature with unusual naevi and teleangiectasiae in 47% of their cases of hemihypertrophy Mental deficiency is found in 28% of the cases There is also a high correlation between renal (Wilms s) (1 2 4) adrenal (5) and hepatic (3) tumours and hemihypertrophy We report a case of congenital hemihypertrophy with acne and folliculitis limited to the hypertrophic side

CASE REPORT

A 16-year-old boy was seen by us because of folliculitis in the scalp and acne in the upper parts of the trunk both on the right side only According to the patient s history his birth was normal after an uneventful pregnancy His birth weight was 4450 g and the Apgar score 10/10 The boy s development was normal At the age of 3 months the mother noticed a difference in temperature between the boy s hands the right being warmer than the left The difference in size between the two sides of his body was noticed later but did not receive much attention

The patient s uncle is said to have large hands and one side of the thorax more elevated but we lack further evidence of hemihypertrophy in his case The other members of the family are healthy

Physical examination revealed a healthy school-boy who measured 180 5 cm in height and 63 kg in weight being at the eighty fourth percentile in the height chart and at the fiftieth in the weight chart for Finnish boys The right side of the body was darker in colour and felt warmer than the left even the superficial veins were more prominent to the right especially on the right ankle Clinically there were no signs of an AV shunt The forehead and the cheekbones were more prominent on the right side and the right shoulder was elevated The extremities measured 1 cm more in length on the right side The maximum difference in circumference of the two sides was 1 5 cm (biceps and thigh) These differences were also demonstrable in radiographs of the extremities Both sides showed a retardation of 2 to 3 years in bone age slightly more on the left side The boy s pubertal stage was expressed as G5 and P5 according to Tanner (196)

On the right side of the patient s head there was mild folliculitis in two areas one over the ear and the other extending from the forehead over the scalp Both areas were constant and about 2 cm broad There was also some acne with multiple comedones and a mild inflammatory reaction on the right upper thorax New lesions were erupting continuously

DISCUSSION

In the present case the only abnormalities were hemihypertrophy with accompanying skin changes Acne and folliculitis were the boy s chief complaints The enlargement of his right arm and leg though previously noted had not been considered severe enough for consulting a doctor Acne was noticed at the age of 14 and is though one sided a problem for the patient The peculiar arrangement of

the folliculitis in segments on the right side of the sc ilp has no explan ition wh itsoever

Heredity is suggested in 7 published cases (2 7) hemihypertrophy occurring mostly in successive gener itions on the m iternal side but this could not be demonstrited in the present c ise

REFERENCES

1 Bjorklund S I Hemihypertrophy ind Wilms s tu mour *Acta Pediatr Scand 44* 287 1955

2 Fraumeni J F Jr Geiser C F & Manning M D Wilms tumor ind congenital hemihypertrophy Report of five new cases and review of literature *Pediatrics 40* 886 1967

3 Geiser C F Baez A Schindler A M & Shih V E Epithelial hep itoblastoma issoci ited with congenital hemihypertrophy and cystathionuria Presentation of a case *Pediatrics 46* 66 1970

4 Miller R W Fraumeni J F & Manning M D Association of Wilms s tumor with aniridia hemihypertrophy and other congenital malformations *N Engl J Med 270* 922 1964

5 Riedel H A Adrenogenital syndrome in a male child due to adrenocortical tumor *Pediatrics 10* 19 19 ?

6 Ringrose R E Jabbour J T & Keele D K Hemihypertrophy *Pediatrics 36* 434 1965

7 Scott A J Hemihypertrophy Report of four cases *J Pediatr 6* 650 1935

Submitted March 21 1979
Accepted May 23 1979

(P K) Department of Paediatrics
Central Hospital of North Karelia
80210 Joensuu
Finland

CASE REPORT

REFLEX SYMPATHETIC DYSTROPHY IN TWO YOUNG FEMALES

G WETTRELL T HALLBÖOK and C HULTQUIST

From the Departments of Paediatrics and Surgery Karnsjukhuset Skovde Sweden

ABSTRACT Wettrell G Hallbook T and Hultquist C (Departments of Paediatrics and Surgery Karnsjukhuset Skovde Sweden) Reflex sympathetic dystrophy in two young females Acta Paediatr Scand 68 923 1979 —Reflex sympathetic dystrophy in paediatric patients is a rarely recognized pain syndrome probably of neurovascular origin The manifestations in two young females consisted of disabling pain and localized hyperesthesia in lower extremities without evident trauma Sympathetic block followed by active mobilization and in the patient with atrophic changes lumbar sympathectomy resulted in complete recovery Reflex sympathetic dystrophy should be considered in the differential diagnosis of pain and tenderness in an extremity

KEY WORDS Pain extremity children

The Reflex Sympathetic Dystrophy syndrome (RSD) is characterized by persistent pain in an extremity It involves mainly hyperaesthesia but vasomotor symptoms and atrophic changes may arise as well (2 7) The condition is often disabling and seems out of proportion when compared with the negligible trauma The syndrome has attracted little attention in clinical paediatric practice and can be misdiagnosed resulting in severe disability (1 3 4 5 6)

This paper describes 2 girls with RSD who were successfully treated with sympathetic blocks in one patient followed by sympathectomy

CASE REPORTS

Case 1

A 14 year-old girl was first seen at the paediatric clinic after having suffered from pain in her right foot for 8 months The pain had started after a period of intense physical activity and immobilization with plaster and analgetics had been futile Physical examination revealed continuous pain up to the level of the malleoli and she could not set weight on her foot There had been some swelling of the toes at first but now atrophy with hyperkeratotic skin and brittle nails dominated She complained of tenderness on the distal plantar surface and even more so localized to a point on the dorsum of the foot The circumference of the right calf was 3 cm less than the left Deep tendon reflexes and peripheral pulses were equal bilaterally

An X ray of the affected foot showed an aseptic partial necrosis in the head of the third metatarsal bone as well as a generalized and marked osteoporosis Measurement of blood flow in the right calf by means of strain gauge occlusion plethysmography showed a slight decrease compared with the left side The blood pressures measured in the ankles were equal and thermography showed no significant difference between sides Laboratory results revealed normal sedimentation rate and serum protein electrophoresis

A lumbar sympathetic block with 0 25% bupivacaine hydrochloride was performed with prompt but incomplete and temporary relief Six days later a right lumbar sympathectomy was done and postoperatively the pain disappeared Thermography showed the right lower leg and foot to be warmer than the left After 9 days of intense physical therapy she was discharged painfree and within a few months she resumed full physical activity the X ray pictures normalized No recurrence of pain has been noted during 1½ year follow up

Case 2

A 9-year-old girl came to the paediatric clinic with a 9 month history of pain localized to the right heel Initially the pain had been intermittent but after 3 months it became continuous For a short period there had been a slight swelling of the foot but no cyanosis For the last

the folliculitis in segments on the right side of the scalp has no explanation whatsoever

Heredity is suggested in 7 published cases (2 7) hemihypertrophy occurring mostly in successive generations on the maternal side but this could not be demonstrated in the present case

REFERENCES

1 Björklund S I Hemihypertrophy and Wilms's tumour Acta Pediatr Scand 44 287 1955
2 Fraumeni J F Jr Geiser C F & Manning M D Wilms tumor and congenital hemihypertrophy Report of five new cases and review of literature Pediatrics 40 886 1967
3 Geiser C F Baez A Schindler A M & Shih V E Epithelial hepatoblastoma associated with congenital hemihypertrophy and cystathioninuria Presentation of a case Pediatrics 46 66 1970
4 Miller R W Fraumeni J F & Manning M D Association of Wilms's tumor with aniridia hemihypertrophy and other congenital malformations N Engl J Med 270 922 1964
5 Riedel H A Adrenogenital syndrome in a male child due to adrenocortical tumor Pediatrics 10 19 19
6 Ringrose R F Jabbour J T & Keele D K Hemihypertrophy Pediatrics 36 434 1965
7 Scott A J Hemihypertrophy Report of four cases J Pediatr 6 650 1935

Submitted March 21 1979
Accepted May 23 1979

(P K) Department of Prediatrics
Central Hospital of North Karelia
80210 Joensuu
Finland

Acta Pædiatr Scand 68 923–924 1979

CASE REPORT

REFLEX SYMPATHETIC DYSTROPHY IN TWO YOUNG FEMALES

G WETTRELL T HALLBÖOK and C HULTQUIST

From the Departments of Paediatrics and Surgery Karnsjukhuset Sko de Sweden

ABSTRACT Wettrell G Hallbook T and Hultquist C (Departments of Paediatrics and Surgery Karnsjukhuset Skovde Sweden) Reflex sympathetic dystrophy in two young females Acta Paediatr Scand 68 923 1979 —Reflex sympathetic dystrophy in paediatric patients is a rarely recognized pain syndrome probably of neurovascular origin The manifestations in two young females consisted of disabling pain and localized hyperesthesia in lower extremities without evident trauma Sympathetic block followed by active mobilization and in the patient with atrophic changes lumbar sympathectomy resulted in complete recovery Reflex sympathetic dystrophy should be considered in the differential diagnosis of pain and tenderness in an extremity

KEY WORDS Pain extremity children

The Reflex Sympathetic Dystrophy syndrome (RSD) is characterized by persistent pain in an extremity It involves mainly hyperaesthesia but vasomotor symptoms and atrophic changes may arise as well (2 7) The condition is often disabling and seems out of proportion when compared with the negligible trauma The syndrome has attracted little attention in clinical paediatric practice and can be mis diagnosed resulting in severe disability (1 3 4 5 6)

This paper describes 2 girls with RSD who were successfully treated with sympathetic blocks in one patient followed by sympathectomy

CASE REPORTS

Case 1

A 14-year-old girl was first seen at the paediatric clinic after having suffered from pain in her right foot for 8 months The pain had started after a period of intense physical activity and immobilization with plaster and analgetics had been futile Physical examination revealed continuous pain up to the level of the malleoli and she ould not set weight on her foot There had been some swell ng of the toes at first but now atrophy with hyperkeratotic skin and brittle nails dominated She complained of tenderness on the distal plantar surface and even more so localized to a point on the dorsum of the foot The circumference of the right calf was 3 cm less than the left Deep tendon reflexes and peripheral pulses were equal bilaterally

An X ray of the affected foot showed an aseptic partial necrosis in the head of the third metatarsal bone as well as a generalized and marked osteoporosis Measurement of blood flow in the right calf by means of strain gauge occlusion plethysmography showed a slight decrease compared with the left side The blood pressures measured in the ankles were equal and thermography showed no significant difference between sides Laboratory results revealed normal sedimentation rate and serum protein electrophoresis

A lumbar sympathetic block with 0 5% bupivacaine hydrochloride was performed with prompt but incomplete and temporary relief Six days later a right lumbar sympathectomy was done and postoperatively the pain disappeared Thermography showed the right lower leg and foot to be warmer than the left After 9 days of intense physical therapy she was discharged painfree and within a few months she resumed full physical activity the X ray pictures normalized No recurrence of pain has been noted during 1½ year follow up

Case 2

A 9 year-old girl came to the paediatric clinic with a 9 month history of pain localized to the right heel Initially the pain had been intermittent but after 3 months it became continuous For a short period there had been a slight swelling of the foot but no cyanosis For the last

the folliculitis in segments on the right side of the scalp h is no explan ition wh itsoever

Heredity is suggested in 7 published cases (2 7) hemihypertrophy occurring mostly in successive gener itions on the m itern il side but this could not be demonstrated in the present c ise

REFERENCES

1 Bjorklund S I Hemihypertrophy and Wilms s tumour *Acta Pediatr Scand 44* 287 1955
2 Fraumeni J F Jr Geiser C F & Manning M D Wilms tumor and congenital hemihypertrophy Report of five new cases and review of literature *Pediatrics 40* 886 1967
3 Geiser C F Baez A Schindler A M & Shih V E Epitheli il hepatoblastoma associated with congenital hemihypertrophy and cystathioninuria Presentation of a case *Pediatrics 46* 66 1970
4 Miller R W Fraumeni J F & Manning M D Association of Wilms s tumor with aniridia hemihypertrophy and other congenital malformations *N Engl J Med 270* 922 1964
5 Riedel H A Adrenogenital syndrome in a male child due to adrenocortical tumor *Pediatrics 10* 19 195
6 Ringrose R E Jabbour J T & Keele D K Hemihypertrophy *Pediatrics 36* 434 1965
7 Scott A J Hemihypertrophy Report of four cases *J Pediatr 6* 650 1935

Submitted March 21 1979
Accepted May 23 1979

(P K) Department of Paediatrics
Central Hospital of North Karelia
80210 Joensuu
Finland

Acta Pædiatr Scand 68 925–927 1979

CASE REPORT

SYSTEMIC AIR EMBOLISM

A Possible Complication of Artificial Ventilation

C E BLANCO L A C RIETVELD and J H RUYS

From the Department of Neonatology and the Department of Radiology University Hospital Leiden The Netherlands

ABSTRACT Blanco C E Rietveld L A C and Ruys J H (Department of Neonatology and Department of Radiology University Hospital Leiden The Netherlands) Systemic air embolism A possible complication of artificial ventilation Acta Paediatr Scand 68 92_ 1979 —A baby of 1100 g and a gestational age of 28 weeks with severe idiopathic respiratory distress syndrome was treated with pressure limited artificial ventilation and positive end expiratory pressure After 30 hours the infant deteriorated and the chest radiograph showed air in the carotid subclavian and aortic artery

KEY WORDS Air embolism artificial ventilation Idiopathic respiratory distress syndrome (IRDS)

In neonatal intensive care units respirators are frequently used for the treatment of idiopathic respiratory distress syndrome (IRDS) This disease requires the use of positive end expiratory pressures in combination with long inspiratory times and sometimes high pressure/volume in order to obtain a reasonable oxygenation and ventilation Such therapy may produce air leaks causing interstitial emphysema pneumothorax or pneumomediastinum Very occasionally it may result in the injection of air directly into the systemic circulation usually with a fatal outcome (1 2)

CASE REPORT

A male baby of 1100 g was born in a general hospital after a gestational period of 8 weeks His Apgar score at 5 min was 1 Respiratory distress developed and immediate continuous positive airway pressure (CPAP) therapy was instituted At 4 hours he was transferred to our specialized unit where he was intubated and connected to a Bourns BP 00 respiratory the peak pressure being set at 35 cmH O and the positive end expiratory pressure (PEEP) at 6 cmH O A chest radiograph at this time showed grade IV hyaline membrane disease (Fig 1) After ?0 hours of ventilation the baby was stable with normal bloodgas values a peak pressure of 22 cmH_2O and a positive end expiratory pressure of 6 cmH O

Then suddenly the baby became cyanotic with a bradycardia and deteriorating bloodgas values A pneumothorax and the obstruction or dislodgment of the endotracheal tube were excluded Crepitations were heard over the whole thorax and the peripheral circulation was markedly reduced Foamy blood started to ooze from the site of the radial artery puncture

A new chest radiograph showed pulmonary interstitial emphysema and intracardiac intraaortic and intrahepatic gas was detected in the X ray

Intravascular gas was also seen in both carotid arteries and both subclavian arteries (Fig 2) The diagnosis of massive arterial air embolism was made The baby died one hour later having survived only 3? hours

DISCUSSION

Air embolism is being recognized as a rare but extremely serious complication of treatment of hyaline membrane disease Only one recent case has been reported as surviving but in that case only venous air embolism was seen (3)

The mechanism resembles lung rupture in connection with free ascent during deep sea diving This is a well known fatal complication of fast decompression resulting from overex

6 months she had been unable to put weight on her right heel and had been walking with a limp

Physical examination demonstrated a hyperesthetic circular area with a diameter of 2 cm on the inner side of the right heel No swelling cyanosis or atrophy of the foot were seen In the lumbosacral region a vascular birth mark was noted Deep tendon reflexes and peripheral sensibility were normal X rays of the calcaneus and of lumbo-sacral regions were normal as were thermographs strain gauge plethysmography and a bone scan of the foot

On the suspicion of RSD a right lumbar block with 0 25% bupivacainechloride was performed with prompt and lasting effect Repeated thermographies showed a transient marked increase in warmth of the right foot Within a month after the block the girl was in full physical activity and during a follow up period of 6 months no pain has recurred

DISCUSSION

Many confusing names (including minor and major causalgia Sudeck atrophy and post traumatic pain syndrome) have been applied to the symptom complex of reflex sympathetic dystrophy (RSD) Today RSD is the preferred name and the syndrome is thought to be a neurovascular disorder (2) The symptoms may occur with latency and after a trauma relatively minor in relation to the severity of the manifestations In some instances the initiating factor is not recognized (1) The pain and hyperesthesia are usually localized to the site of injury Sometimes the pain progresses proximally without being confined to a peripheral nerve distribution This confuses the physicians who suggest a psychiatric component Severe degrees of the syndrome also seem to occur more frequently in patients with certain personality traits (1 7)

During the early stages vasomotor disturbances are often manifested by signs of vasodilation Later vasospastic symptoms dominate with coldness pallor or cyanosis Atrophic changes involving skin and nails develop insidiously (2) but are seldom seen in paediatric patients (1) The differential diagnosis may include peripheral vascular disorder or peripheral neuritis osteomyelitis and tenosynovitis

Therapy for RSD should be instituted promptly to improve the possibility of permanent relief In the early or mild stages conservative treatment with analgetics warmth and physical therapy may be of value (1) In the younger age group a more active attitude with diagnostic blocks and in the severe stages sympathectomy is often preferred (2) Recently treatment of a child by transcutaneous nerve stimulation has been reported (6) After relief of pain active exercise is important in the process of rehabilitation

REFERENCES

1 Bernstein B B Singsen B H Kent J T Kenreich H King K Hicks R & Hanson V Reflex neurovascular dystrophy in childhood *J Pediatr* 93 211 1978
2 Bonica J J Causalgia and other reflex sympathetic dystrophy *Postgrad Med* 53 143 1973
3 Fermaglich D R Reflex sympathetic dystrophy in children *Pediatrics* 60 881 1977
4 Guntheroth W G Chakmakjian S Brena S C Ricketts H J & Wiederhielm C A Posttraumatic sympathetic dystrophy *Am J Dis Child* 121 511 1971
5 Kozin F Haughton V & Ryan L The reflex sympathetic dystrophy in a child *J Pediatr* 90 417 1977
6 Richlin D M Carron H Rowlingson J C Sussman M D Baugher W H & Goldner R D Reflex sympathetic dystrophy Successful treatment by transcutaneous nerve stimulation *J Pediatr* 93 84 1978
7 Wirth F R & Rutherford R B A civilian experience with causalgia *Arch Surg* 100 633 1970

Submitted March 3 1979
Accepted April 9 1979

(G W) Department of Paediatrics
Karnsjukhuset
S 541 01 Skovde
Sweden

Acta Pædiatr Scand 68: 925–927, 1979

CASE REPORT

SYSTEMIC AIR EMBOLISM

A Possible Complication of Artificial Ventilation

C. E. BLANCO, L. A. C. RIETVELD and J. H. RUYS

From the Department of Neonatology and the Department of Radiology, University Hospital, Leiden, The Netherlands

ABSTRACT. Blanco, C. E., Rietveld, L. A. C. and Ruys, J. H. (Department of Neonatology and Department of Radiology, University Hospital, Leiden, The Netherlands). Systemic air embolism. A possible complication of artificial ventilation. Acta Paediatr Scand, 68: 925, 1979.—A baby of 1100 g and a gestational age of 28 weeks with severe idiopathic respiratory distress syndrome was treated with pressure limited artificial ventilation and positive end expiratory pressure. After 30 hours the infant deteriorated and the chest radiograph showed air in the carotid, subclavian and aortic artery.

KEY WORDS: Air embolism, artificial ventilation, idiopathic respiratory distress syndrome (IRDS)

In neonatal intensive care units respirators are frequently used for the treatment of idiopathic respiratory distress syndrome (IRDS). This disease requires the use of positive end expiratory pressures in combination with long inspiratory times and sometimes high pressure/volume in order to obtain a reasonable oxygenation and ventilation. Such therapy may produce air leaks causing interstitial emphysema, pneumothorax or pneumomediastinum. Very occasionally it may result in the injection of air directly into the systemic circulation, usually with a fatal outcome (1, 2).

CASE REPORT

A male baby of 1100 g was born in a general hospital after a gestational period of 28 weeks. His Apgar score at 5 min was 1. Respiratory distress developed and immediate continuous positive airway pressure (CPAP) therapy was instituted. At 4 hours he was transferred to our specialized unit where he was intubated and connected to a Bourns BP 200 respiratory, the peak pressure being set at 35 cmH$_2$O and the positive end expiratory pressure (PEEP) at 6 cmH$_2$O. A chest radiograph at this time showed grade IV hyaline membrane disease (Fig. 1). After 20 hours of ventilation the baby was stable, with normal bloodgas values, a peak pressure of 22 cmH$_2$O and a positive end expiratory pressure of 6 cmH$_2$O.

Then suddenly the baby became cyanotic with a bradycardia and deteriorating bloodgas values. A pneumothorax and the obstruction or dislodgment of the endotracheal tube were excluded. Crepitations were heard over the whole thorax, and the peripheral circulation was markedly reduced. Foamy blood started to ooze from the site of the radial artery puncture.

A new chest radiograph showed pulmonary interstitial emphysema, and intracardiac, intraaortic and intrahepatic gas was detected in the X-ray.

Intravascular gas was also seen in both carotid arteries and both subclavian arteries (Fig. 2). The diagnosis of massive arterial air embolism was made. The baby died one hour later, having survived only 32 hours.

DISCUSSION

Air embolism is being recognized as a rare but extremely serious complication of treatment of hyaline membrane disease. Only one recent case has been reported as surviving but in that case only venous air embolism was seen (3).

The mechanism resembles lung rupture in connection with free ascent during deep sea diving. This is a well known fatal complication of fast decompression resulting from overex



Fig 1 Patient aged 4 hours IRDS grade IV

pansion of the lungs (4) The sequence of events is as follows In an emergency rise to the surface during a dive with compressed air the diver develops a glottis cramp which prevents expiration Thus air is trapped in the lungs which then blow up like balloons to much greater than their normal size (e g 1 volume unit of air at 5 atmospheres tends to enlarge to 5 times its size at surface atmospheric pressure)

If the lungs expand to the extent that the alveoli burst then the air is sucked into the pulmonary venous system and by way of the left ventricle to the systemic circulation This same situation can be reproduced at a pressure of 10 6 kPa (80 mmHg) (5) Without proper treatment this condition is fatal For the diver treatment consists of immediate re*compression* with higher pressure Cabarrou (6) has recommended recompresssion in a chamber to 15–20 atmospheres and ventilation with an oxygen helium mixture to reduce the gas bubble size in the circulation as quickly as possible The patient should be placed in Trendelenburg position which serves to reduce the risk of air bubbles reaching the head and causing brain damage

Of course the premature infant is not exposed to the same high pressure situation as adult divers Nevertheless the infant s alveoli can be overexpanded during treatment with assisted ventilation The *mechanism* then seems to follow a similar course In neonatal medicine one should be aware of this complication and recognize it radiographically Whenever long inspiratory times and PEEP are used with high pressure/volume and particularly when interstitial emphysema is present there is a high risk of air embolism Signs to look for are changes in the peripheral circulation consisting of well defined migratory areas of pallor or sudden deterioration of the patient s condition without an obvious explanation

In the event of a suspected air embolism treatment possibilities are limited Changing *the setting or even temporarily discontinuing* the artificial ventilation may improve the situation If the respirator is stopped however the infant most probably will become hypoxic in which case treatment with a pulmonary arterial vasodilator (Tolazoline) may be useful (7)

Massive air embolism should be considered as a cause of death in prematures with IRDS treated by positive pressure ventilation It may be that the principles of treatment for air embolism in diving medicine could be re evaluated for possible application to the care of newborns

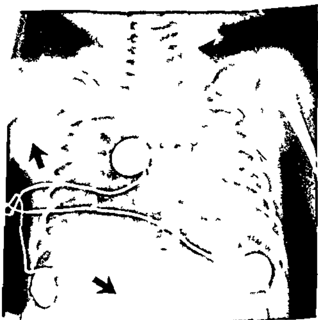


Fig 2 Patient aged 31 hours Arterial air embolism

REFERENCES

1 Henderson Smart D J Mas ive air embolism in a neonate with respiratory distress *Med J Aust 2* 641 1975
Siegle R L Eyal F G & Rabinowitz J G Air embolus following pulmonary interstitial emphysema in hyaline membrane disease *Clin Radiol* ?7 77 1976
3 Kogutt M S Systemic air embolism secondary to respiratory therapy in the neonate six cases including one survivor *Am J Roentgenol 131* 4 5 1978
4 Kinsey J L Air embolism as a result of submarine escape training *US Armed Fo ces Med J 5* ?43 1954
5 Ehm O F *Tauchen – noch sicherer* Albert Muller Verlag Zurich 1974
6 Cabarrou P Der Dekompressionsunfall und seine Be handlung *Munch Med Wochenschr 108* 155? 1966
7 Goetzman B W Sunshine Ph Johnston J D Wennberg R P Hackel A Merten D F Bartoletti A L & Silverman N H Neonatal hypoxia and pulmonary vasospasm Response to tolazoline *J Pe diatr 89* 617 1976

Submitted Febr .0 1979
Accepted April 3 1979

(C E B) Department of Neonatology
University Hospital
Rijnsburgerweg 10
Leiden
The Netherlands

NEW BOOKS RECEIVED

A G Coran D M Behrendt W H Weintraub & D C Lee *Surgery of the Neonate* 77? pp illus Little Brown & Co Boston Massachusetts 1978 $3? 50 ISBN 0-316-15635 3

F Falkner & J M Tanner (eds) *Principles and Prenatal Growth* Vol 1 *In* Human Growth 633 pp illus Ballière Tindall London 1978 £ 4 75 ISBN 0 70 0 0731 5

F Falkner & J M Tanner (eds) *Postnatal Growth* Vol II *In* Human Growth 634 pp illus Ballière Tindall London 1978 £ 4 75 ISBN 0-70_0-073? 3

V Dubowitz *Muscle Disorders in Childhood* Vol XVI *In* Major Problems in Clinical Pediatrics Saunders London and Philadelphia 1978 ?8? pp illus No price given ISBN 0-7 16-3 10-6

K Roberts *Manual of Clinical Problems in Paediatrics with annotated key references* 461 pp Little Brown & Company Boston Massachusetts 1979 $10 95 ISBN 0-316 74984

J H Jonxis (ed) *Growth and Development of the Full term and Premature Infant* The Jonxis Lecture Volume I Excerpta Medica Amsterdam 1978 293 pp illus $31 00 ISBN 0-444 90050-0

E L Kendig Jr & V Chernick (eds) *Disorders of the Respiratory Tract in Children* 3rd ed W B Saunders Company London 1115 pp illus £3 50 ISBN 0-7?16-5378 ?

G Stamatoyannopoulos & A W Nienhuis (ed) *Cellular and Molecular Regulation of Hemoglobin Switching* 792 pp illus Grune & Stratton Inc 1979 $68 50 ISBN 0 8089 1159 7

M Miskin (ed) *Ultra Sound in Paediatrics* 337 pp illus Grune & Stratton Inc New York 1979 $?9 50 ISBN 0 8089 1117 1

F Falkner & J M Tanner (eds) *Neurobiology and Nutrition In* Human Growth 3 606 pp illus Plenum Press New York 1979 $37 50 ISBN 0 306 34463 7

M Parsons *Tuberculous Meningitis* A handbook for clinicians 83 pp illus Oxford Medical Publications London 1979 £4 50 ISBN 0 19 261166 6

L A Barness *Advances in Paediatrics* Vol 25 510 pp Year Book Medical Publishers London 1978 $45 25 ISBN 0 8151 0497 9

C G Geary *Aplastic Anaemia* 249 pp illus Ballière Tindall London 1979 £1 50 ISBN 0 70?0 0698 X

P H Morris Jones (ed) *Topics in Paediatrics 1 Haematology and Oncology* 156 pp illus Pitman Medical Tunbridge Wells England 1979 £9 50 ISBN 0-272 79540-?

BOOK REVIEWS

A. K. K. Kreutner & D. R. Hollingsworth: *Adolescent obstetrics and gynecology.* Year Book Medical Publishers Inc. Chicago London 1978. 658 pp. illus. $50.50. ISBN 0-8151-5200-0

This textbook, which is intended for physicians, especially obstetricians and gynecologists, students and paramedical professionals, is a useful approach to common obstetrical and gynecological problems of adolescence. Many chapters are relevant only to teenagers but other topics have a broader application.

The first part of the book is a review of adolescent sexuality and an excellent presentation of the hypothalamic-pituitary-gonadal system. The section that follows is concerned with the maturation of this system during puberty, from its quiescent state in the child to its fully operational state in the adult.

Many chapters deal with teenage pregnancies. The discussion about abortion and abortion counselling is up to date and well suited to the conditions in Sweden since the new law on abortion. The fact that the teenager alone has to decide about pregnancy is emphasized. The counsellor's role is to supply necessary information about alternatives, explain the details of any procedures and provide contraceptive information. Pregnant adolescents also require specialized prenatal care because of their age and socio-economic circumstances. It is therefore more important to use the assistance of social workers than to give a too detailed description of the nutrition during pregnancy with dietary tables and sample menus.

Pre-eclampsia often occurs in teenage patients. The authors present a useful clinical guideline for the management of hypertensive patients. Early diagnosis and adequate treatment may prevent severe progression of the disease. Pregnant diabetic adolescents run an increased risk of pre-eclampsia. Identification of gestational diabetes is therefore important and a simple classification is presented and the therapeutic principles discussed.

Numerous studies have shown that many women—and especially teenagers—take a great many drugs during pregnancy. Since some drugs have been shown to be potentially teratogenic the authors give a comprehensive review of the most commonly ingested drugs. The book ends with detailed appendices on drugs and adolescent pregnancy.

Dysmenorrhea is the most common gynecological complaint of the adolescent. The extensive use of oral contraceptive agents has reduced its frequency, but many adolescents do not like or need such hormone therapy. New therapeutic possibilities have been obtained with the different kinds of prostaglandin inhibitors.

The question of birth control and the efficacy and side effects of the various methods are briefly discussed. In contrast to the authors, we in Sweden never support the opinion that intra-uterine devices might be the contraceptive method of choice for the sexually active adolescent.

The chapter concerning breast disorders is very important and deals in an excellent way with all the problems for which an adolescent can consult a doctor. True gynecological neoplasms in teenagers are uncommon. Two-thirds of them develop in the ovary. Since most of the ovarian neoplasms are germ cell tumours they are unilateral, encapsulated and seldom give peritoneal metastases. This often makes conservative surgery possible and thus also preserved reproductive capacity. The increasing frequency of vaginal and cervical clear cell adenocarcinoma in adolescent girls exposed to intrauterine diethylstilbestrol prior to 10–16 weeks of gestation is discussed too. A large number of illustrations, tables and references enhance the value of the book, which can be highly recommended.

Lars Svanberg

R. Mande, N. Masse & M. Manciaux (eds.): *Pédiatrie sociale.* 2nd ed. 715 pp. Flammarion Médecine Sciences, Paris 1977. No price given. ISBN 2-257-20381-X

The teaching of basic social paediatrics is or should be included in the ordinary curriculum at medical school. In a few countries there are special graduate or postgraduate courses in social paediatrics, in Sweden for example a one week compulsory course for specialization in paediatrics. Since the reorganization of the National Health Service in Britain, the number of community paediatricians has increased rapidly, and recently a university degree of Master of Medical Science in Community Paediatrics has been proposed.

In the USA a 2 year training program in social or community paediatrics is now offered at six universities. The purpose is that these paediatricians later will enter medical faculties and serve as "infiltrators" of socio-paediatric ideas.

The greatest experience in socio-paediatric teaching, however, comes from France, where the Centre International de l'Enfance every year since 1950 has organized international courses in social paediatrics. At some medical schools in France there are even special chairs of social paediatrics.

Therefore it is not surprising that the first and so far the only major European text book on social paediatrics should appear in French. The first edition appeared in 1972 and now a second extended one has come out. The editors are French paediatricians, well known in international socio-paediatric and educational circles. They have succeeded in making the contributors produce a very comprehensive and rich volume. The five parts of the book deal with General aspects, including demographic and bio-statistic aspects of childhood, The Normal Child, The Adolescent, Social Aspects of Childhood diseases and Organization of Preventive Care. Although the experiences and points of view are mainly French, there is

o much of generally applicable knowledge and informa ion that the book us useful also for a non French reader t is not possible to mention all the good parts of the ook There is for instance a good chapter on vaccina ions dealing in detail with all important childhood vac inations and their indications and complications In the next edition the text on smallpox vaccination could per haps be cut down to just one page or so

Other parts which I have found especially valuable are those on caries accidents and chronic diseases

In the chapter on perinatal problems the emphasis is put on prematurity and its socio-economic causes I think the importance of the family s socio-economic status for the ultimate outcome of the premature baby should be discussed more fully

Curiously enough I could not find anything at all about the newborns injured by their mothers abuse of alcohol during pregnancy This so-called foetal alcohol syndrome is rapidly developing into a first rate socio paediatric problem not only in France where it was first described in modern time but in all industrialized countries

Altogether it is a valuable and well composed book and it should be welcomed by students nurses and pae diatricians since it gives them a full view of social pae diatrics and material for new thoughts

In one aspect however it is like almost all other text books there is little criticism of conventional medical procedures treatment and prevention It is a pity that the knowledge and experience of sociologists and psychol ogists are not used more extensively As far as I can see almost all the authors are doctors I also miss a critical analysis of the numerous screening procedures that are carried out in preventive paediatrics i e a basic meth odological discussion on evaluation principles In my ex perience this type of biostatistics is a very useful tool for a social paediatrician whether he is reading or writing ar ticles and it is rarely taught in general paediatrics By introducing a chapter on epidemiology and cutting down the chapter on French administration and organization the book would be made still more useful internationally

Lennart Kohler

V Dubowitz *Muscle diso de s in ch ldh od* Vol XVI *In* Major problems in clinical pediatrics Saunders London and Philadelphia 1978 8 pp illus No price given ISBN 0-7 16-3 10-6

In the pref ce to his book Victor Dubowitz says that the cl nician s place is not only at the bedside but also in the laboratory in order to get a comprehensive over view of the patient and his investigations and not only try to draw conclusions from either alone

This book gives the essence of twenty years experience of children with muscle disorders at the bedside com bined with the clinically relevant parts of the previous laboratory book *Muscle Biopsy A Modern Approach* by Dubowitz and M H Brooke (1973)

In addition to muscle disorders in the narrow sense of the word there are chapters dealing with disorders of the lower neuron myasthenia gravis inflammatory myo pathies disorders with muscle contracture and joint rigidity and disorders of movement

Professor Dubowitz has combined his broad experience as a clinician and scientist with his talent as a writer and teacher The result is a splendid manual in the art of mak ing the diagnosis in the entangled field of neuromuscular disorders It will become the first choice for reference for the practising pediatrician as well as for the neurolo gist and orthopedic surgeon

O Hansson

J H Jonxis (ed) *Grow th and development of the full term and premature infant* The Jonxis Lectures Volume 1 Excerpta Medica Amsterdam 1978 ?93 pp illus US $31 00 ISBN 0-444 90050-0

This volume contains a collection of lectures given to the Postgraduate Course on Growth and Development of the Full term and Premature Infant held in Curaçao from 21 to ?3 November 1977 The presentations are given by in ternationally well known European and American scien tists within the field of neonatology The various contri butions deal with topics of current interest such as re gulation of growth and nutrition of the fetus and nutri tional needs energy balance and metabolism of the new born All individual papers are of high scientific quality and contain a rather comprehensive presentation of the topic In the last chapter Dr Widdowson in a very sti mulating way gives comments on the various presen tations

The book can be recommended to all those who want to read a condensed presentation of current progress in some of the most important fields of neonatology

Rolf Zetterstrom

ANNOUNCEMENT

The Fifth World Symposium on Pediatric Surgery will be held from the 15th to the 19th of October 1980 at the Acapulco Princess in Acapulco Congress office Insurgentes Sur 3700 Mexico 22 D F Mexico